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MALE STERILITY IN MEDICAGO SATIVA L.[1]

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A wide variation between plants in the percentage of aborted pollen is readily observable in alfalfa. Partial male sterility occurs frequently, whereas complete male sterility is rare. The term "male sterility" is used because there is no corresponding impairment in female fertility in these plants. Plant breeders concerned with seed production were early impressed with the frequency of occurrence and constancy of the pollen abortion character.

Seed production in alfalfa results almost entirely from cross pollination. Specific factors causing pollen abortion, therefore, could be carried into succeeding generations mainly through the maternal source. Hence, there is no rigid selection against male sterile factors such as would obtain in a self-pollinated species. Presumably, random sampling of pollen incurred through insect pollination would tend to reduce the importance of aborted pollen as a limiting factor in seed production.

The following approach to the study of the problem of pollen abortion in alfalfa includes its cytological basis, nature of inheritance and effect on self- and cross-fertility.

LITERATURE REVIEW

Clarke and Fryer (5) observed, that at intervals of several days within a single season, and over a three-year period, the percentage of aborted pollen grains in selected alfalfa plants was relatively constant. A study of pollen sterility of 36 alfalfa plants analysed in two successive winters was reported by Brink and Cooper (4). They obtained a correlation coefficient between pollen sterility readings in two winters of $r = .61$.

Clarke and Fryer (5), Engelbert (7), Brink and Cooper (4) and Bolton and Fryer (3) reached the general conclusion that varying percentages of pollen sterility may have an appreciable effect on seed-setting in some cases. Armstrong and White (1) believed that pollen sterility was a factor which influenced pod-setting and the number of seeds per pod and that a high percentage of shrunken pollen grains seemed to be associated with faulty dehiscence of the anthers. Armstrong and White (1) analysed pollen sterility in eight F_2 progeny obtained from selfing a hybrid plant and concluded that there was definite segregation of a factor or factors responsible for pollen sterility.

Complete male sterility controlled by heritable factors has been reported in higher plants by numerous workers. Plants possessing complete male sterility, inherited as a simple recessive condition, have been reported

[1] Contribution from the Division of Forage Crops, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada. A thesis submitted to the Graduate School of the University of Wisconsin in partial fulfilment of the requirements of the degree of Doctor of Philosophy.

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in *Viola* by Clausen (6), in *Pisum* by Fabergé (8) and in *Lycopersicum* by Rick (13). Gustafsson (11) has reviewed the problem of the degeneration of meiosis in apomictic species, covering many aspects of the problem including the effect of the tapetal development upon the course of meiosis. Gates (9), Gates and Thomas (10) and Oehlkers (12) studied male sterile plants of *Oenothera Lamarckiana* in which tapetal disturbances were associated with the male sterile condition. Clausen (6) in *Viola*, Artschwager (2) in *Brassica* and Rick (13) in *Lycopersicum* have described atypical microsporogenesis in selected male sterile plants. They reported tapetal disfunction as a cause of the breakdown of microsporogenesis.

MATERIALS AND METHODS

The plants selected for these studies were obtained from cultures at the Genetics Department of the University of Wisconsin. Two plants, one completely male sterile and one fully male fertile, of unknown genetic constitution otherwise were selected as parent material. The completely male sterile plant chosen for this study was found later to contain other factors for partial pollen sterility. Hybrid seed was obtained on the male sterile plant. An F_1 progeny was then grown and analysed. Subsequently second generation progenies were grown from F_1 plants differing in per cent aborted pollen. The self- and cross-fertility relationships of sister plants differing in per cent aborted pollen were determined. A description of the critical stages in microsporogenesis of these sister plants was made.

The complete male sterility of the plant P-1-3 was the principal reason for choosing it as a parent in this experiment. The plant 50–46 had less than 6 per cent aborted pollen and was selected as the male parent. A cross was made between the above plants, the resulting seed being harvested in the spring of 1948. F_1 seedlings were established under greenhouse conditions at Ottawa, Ontario, September 1948, to the number of 100, and 53 additional seedlings were transplanted to the field in mid-October.

Six of the F_1 plants grown under greenhouse conditions, and varying in percentage aborted pollen from 5 to 81 per cent, were selected for cytological studies of microsporogenesis. The following August one completely male sterile and a male fertile (4.5 per cent aborted pollen) check plant were selected from the F_2 progeny of one F_1 plant, H-8, for a comparative cytological study of microsporogenesis.

The 100 F_1 plants were transplanted to the field in the first week of June. F_2 progenies, from plants varying in percentage aborted pollen from 5.5 to 71 per cent, were established in the greenhouse and later transplanted to the field during the first week of June.

All material involved in the cytological investigation of microsporogenesis was prefixed in Carnoy's and then placed in a modification of Karpechenko's fixative. Serial sections were cut at 12 microns and stained in Delafield's hematoxylin followed by safranin.

Pollen was sampled in order to obtain percentage aborted pollen data for each plant. The method of sampling pollen was as follows: three florets were tripped into a drop of aceto-carmine and a coverslip was placed in position. Two traverses were made, from top to bottom and from left to right, the stained and unstained pollen grains were counted under high dry

magnification (10 × 40). The stained grains were recorded on a Veeder counter and the unstained numbers were recorded mentally. The total number of grains counted varied from 120 to 600. The two classes, stained and unstained pollen grains, were discrete (Figure 1).

Two series of pollen counts were made from (A) open flowers on the first raceme to bloom, and from (B) such open flowers as were on the plant nine days later. The 100 F_1 plants were analysed in this manner as they came into bloom.

A statistical analysis was made to test the effect of size of sample upon the percentage aborted pollen figures. Eight F_1 plants of varying per cent male sterility were chosen, and samples of 40, 80, 320 and 640 pollen grains were taken and the per cent aborted pollen calculated on each. The analysis of variance indicated no significant difference for size of sample. The field-sampled male sterility data were all calculated on the basis of a 200 pollen grain sample.

The potato leaf hopper *Empoasca fabae* (Harr.) was present in epidemic proportions from mid-July until late August causing some stunting and yellowing of the plants. F_2 plants which failed to bloom under these conditions were cut back and brought into the greenhouse where under controlled conditions the majority of the plants eventually flowered. It was found that there was no appreciable difference in male sterility due to the change in environmental conditions as tested by the means of field and greenhouse data.

Some plants in the F_2 progeny were observed in which pollen was entirely lacking. Whole mounts of anthers were made by the method outlined by Thirumalachar (14) using lactophenol-acid fuchsin and later lactophenol-analine blue. Those plants in which the anthers were empty and shrunken were designated non-dehiscent. All plants which were not completely male sterile were classified as partially male sterile.

RESULTS

The results of the investigation are divided into two parts—one part concerned with factors causing a complete male sterile condition, and the other part concerned with factors causing a partial pollen abortion. The parent plants were observed to have a chromosome number of $2n = 32$, the normal value for the species.

Complete Male Sterility

Cytological Observations

Microsporogenesis in the female parent P-1-3 was extremely irregular. An early degeneration of the pollen mother cells led to the ultimate production of empty, shrunken anthers at maturity. A regular pattern of microsporogenesis leading to the production of approximately 95 per cent plump pollen grains occurred in the male parent.

Two plants, H-8-99 and H-8-74, were chosen from the F_2 progeny of H-8 in order to investigate the recurrent parental types. An anther from male fertile H-8-99 was filled with plump pollen grains (Figure 2 A), whereas an anther from male sterile H-8-74 was shrunken and devoid of pollen grains (Figure 2 B).

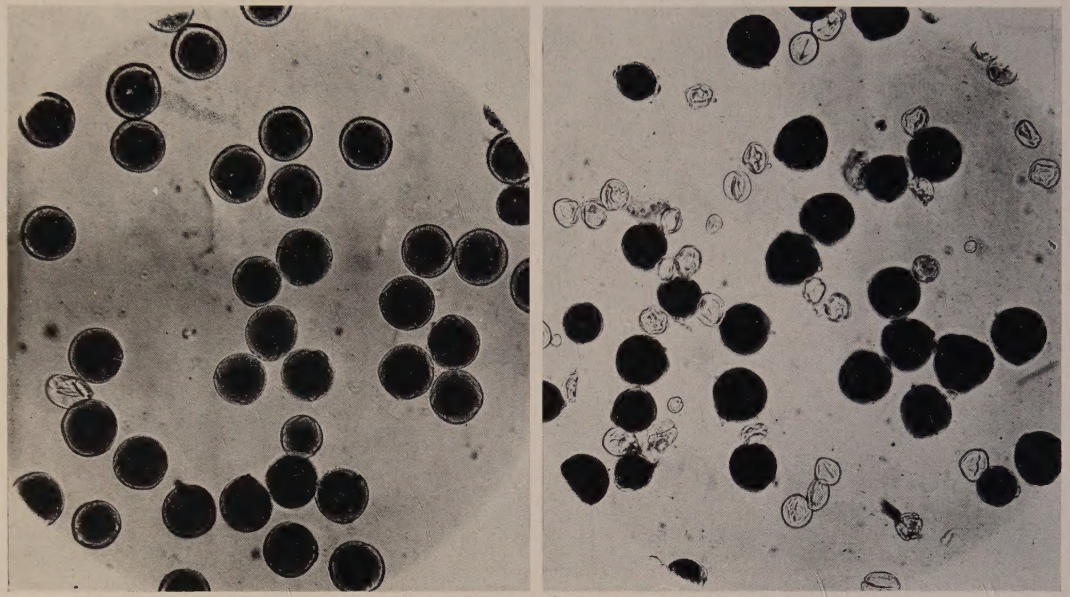
It is generally accepted that a close relationship exists between the development of the anther tapetum and the course of microsporogenesis. In alfalfa, the cells of the tapetum remain uninucleate throughout their development and are characterized by larger nuclei and a more densely staining cytoplasm than those of the surrounding tissues.

In the anthers of the pollen fertile plant H-8-99, the sporogenous and adjacent tapetal tissue were not sharply differentiated by their cytoplasmic density in the period up to and just previous to the onset of meiosis. During the period in which the nuclei of the microspore mother cells were passing from the zygotene to the diakinesis stage, the nuclei of the adjacent tapetal cells increased in size and contained characteristically large prochromosomes (Figure 2 C). The tapetal cells also increased in cytoplasmic density during this period. The size and shape of the tapetal cells remained relatively unchanged until the microspores separated from their tetrads. Thereafter the tapetal cells and their nuclei became elongate and flattened (Figure 2 E). As maturation of the pollen grains approached, the tapetum was completely absorbed so that finally the wall of the locule was composed of a thick walled endothecial layer (Figure 2 G).

The behavior of the tapetum in the anthers of the completely male sterile plant H-8-74 was in sharp contrast to that just outlined for the pollen fertile plant. Just previous to the onset of meiosis the tapetum layer became considerably swollen and densely cytoplasmic; whereas the adjacent sporogenous cells were characterized by extreme vacuolation of their cytoplasm. These degenerative changes which took place in the anthers showed great variability in respect to time of onset, but as the swelling and ingrowing tapetum enlarged, the sporogenous tissue passed through extreme vacuolation of the cytoplasm, and disintegration of the cell walls and nuclear membranes proceeded until only the nucleoli and oil-like droplets were present. The extreme swelling of the tapetum and characteristic early degenerative symptoms occurred in anthers in which the pollen mother cells appeared to be in the early zygotene stage (Figure 2 D). The residual elements of some disintegrating pollen mother cells consisted of nucleoli and weakly staining nuclear material (Figure 2 F). Metaphase I stages were observed in several cells of the same anther, in some cases, and in such anthers the tapetal tissue was not swollen and hence simulated the normal behaviour. The tapetum continued to remain densely cytoplasmic following the complete disintegration of the pollen mother cells and in some cases filled approximately 70 per cent of the locule. Later, the tapetum disintegrated leaving the locules of the anthers filled with large oil-like degeneration products. Some heavy walled pollen grains were present in a few cases at the maturation of the anthers (Figure 2 H). However, the shrunken nature of the anthers would preclude the possibility of anthesis.

Inheritance Studies

The inheritance studies were based on a classification of the plants into dehiscent (male fertile) and non-dehiscent (male sterile) classes. The F_1 generation, represented by 149 plants, was dehiscent. The distribution of variates in the F_2 families is summarized in Table 1. In the progeny of H-8 and H-57 the chi-square values indicated a good fit to a 3 : 1 ratio for



H–64
3.5% Aborted Pollen

H–85
55% Aborted Pollen

FIGURE 1. Photomicrographs of representative fields of pollen grains × 180.

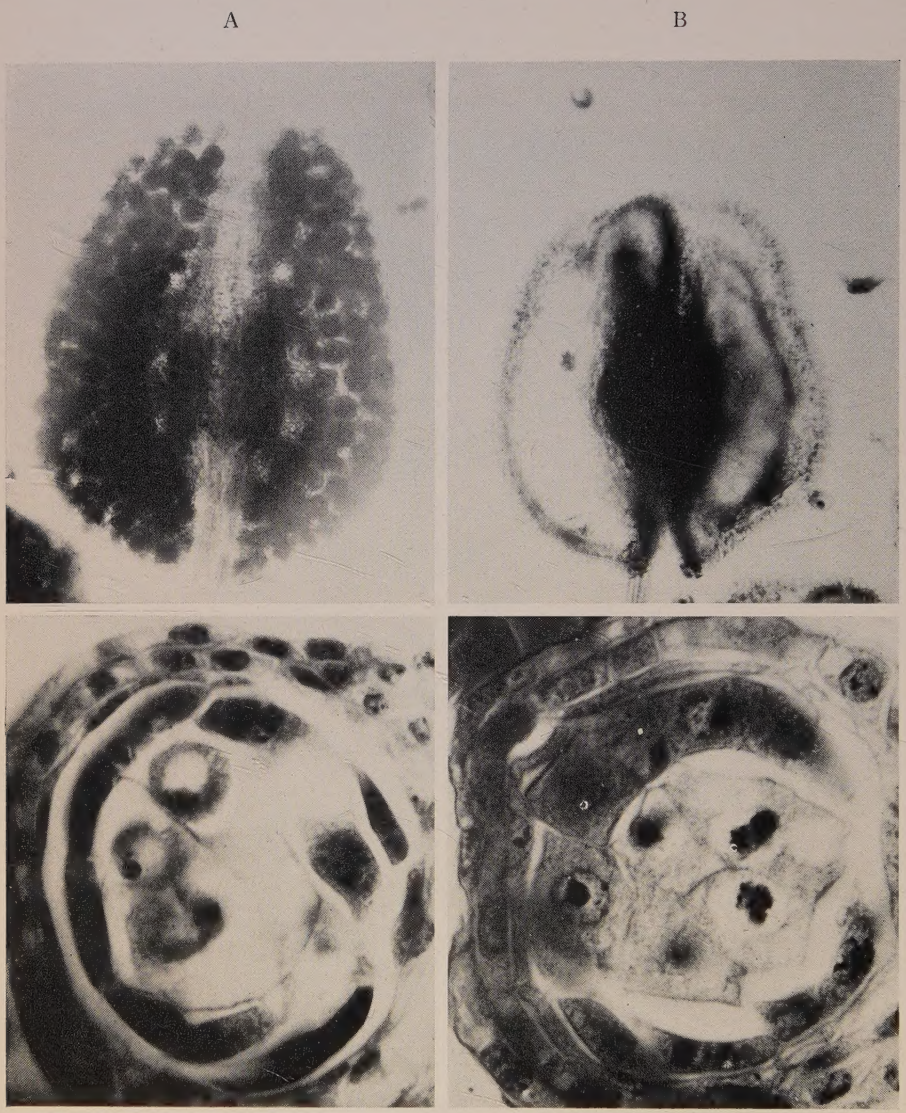
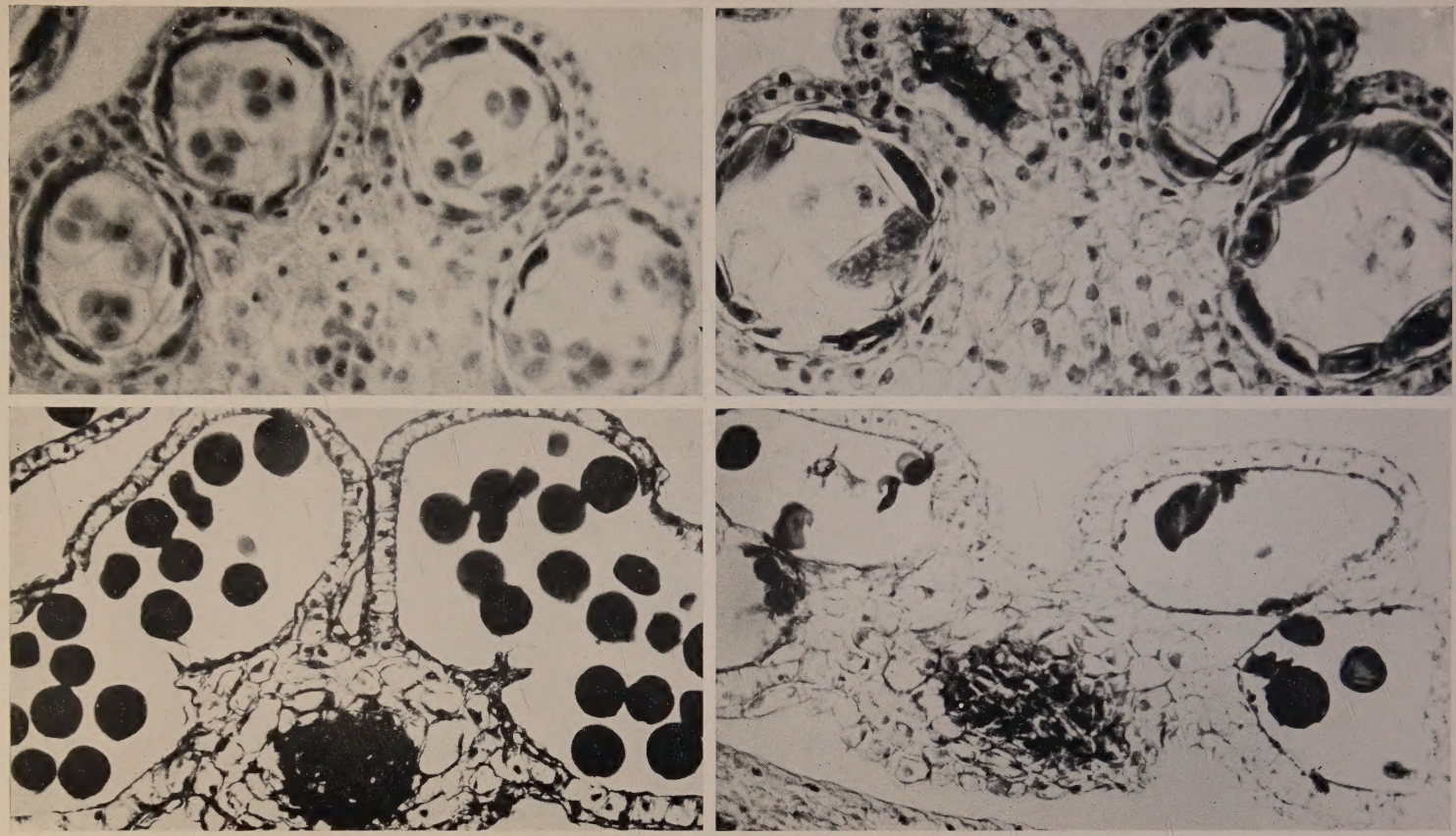


FIGURE 2. A and B, whole mounts of anthers (× 90) C and D, cross-sections of locules showing tapetal layer and early prophase stages × 1100.

E × 400 F



G × 260 H

H–8–99 H–8–74

FIGURE 2. E and F, cross-section of anthers, at approximately the tetrad of microspore stage × 400. G and H, cross-section of anthers just previous to anthesis × 260.

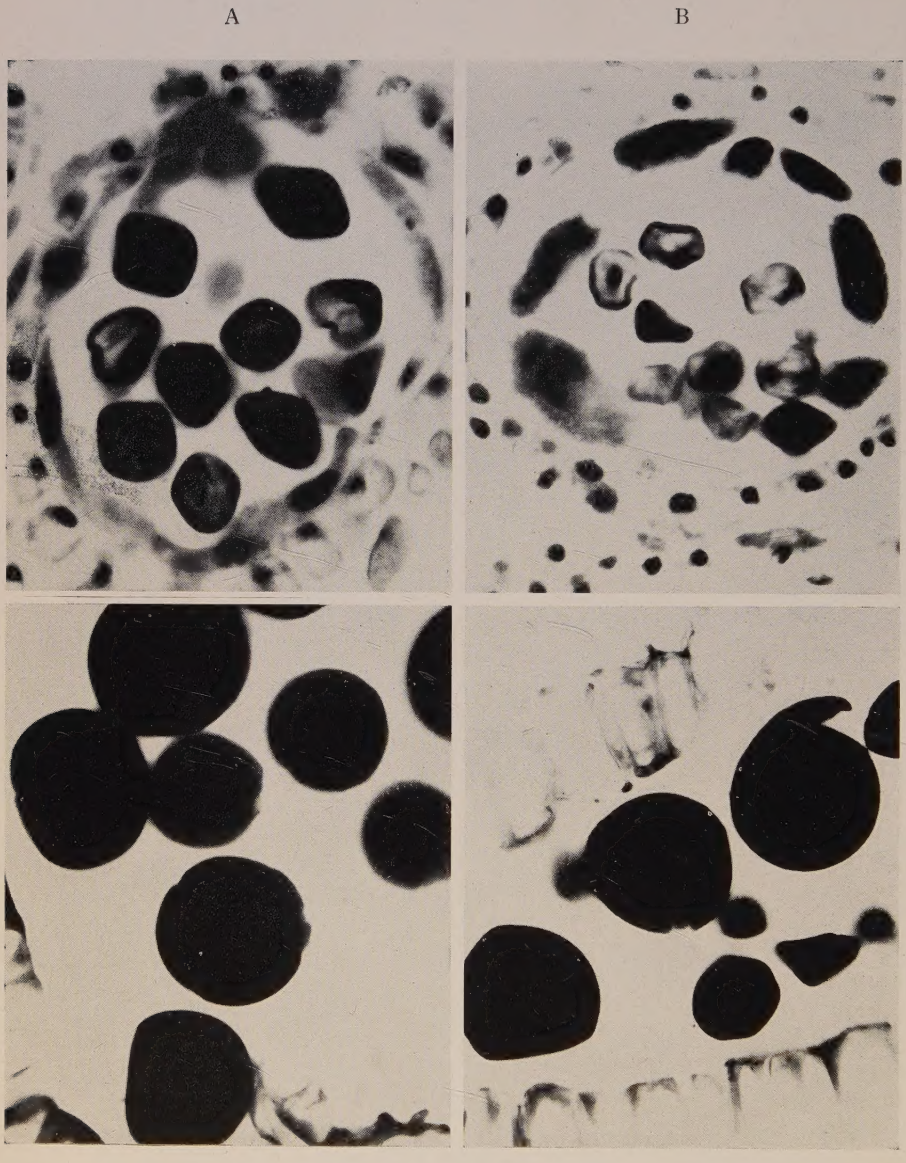


FIGURE 3. A and B, cross-sections of locules showing microspores and tapetal tissue. C and D, cross-sections of locules just previous to anthesis. Photomicrographs × 820.

TABLE 1.—TEST FOR GOODNESS OF FIT IN F_2 FAMILIES OF P–1–3 × 50 — 46 TO 3 : 1 AND 15 : 1 RATIOS RESPECTIVELY

F_2 Family	Dehiscent		Non-dehiscent		Total	χ^2	Ratio Fitted
	Obs.	Calc.	Obs.	Calc.			
H–8	143.0	143.5	47.0	46.5	190.0	.054*	3 : 1
H–57	42.0	42.8	15.0	14.2	57.0	.065	3 : 1
H–71	65.0	66.6	6.0	4.4	71.0	.62	15 : 1
H–74	86.0	86.3	6.0	5.7	92.0	.31	15 : 1
H–46	103.0	95.9	10.0	7.1	113.0	1.88	15 : 1
H–85	78.0	81.6	9.0	5.4	87.0	2.56	15 : 1
H–3	22	21.6	1.0	1.4	23.0	.18	15 : 1

* In this table the data for each F_2 family indicate no significant deviations from the fitted ratios.

the fertile : sterile character. Similarly, in the progenies, H-71, H-74, H-46, H-35 and H-3 the chi-square values indicated a good fit to a 15 : 1 ratio. An F_2 progeny of H-13 consisting of 234 plants did not segregate any completely male sterile plants but all showed the dehiscent character.

Partial Male Sterility

Cytological Observations

Meiosis was regular in six F_1 plants varying in percentage aborted pollen from 5.5 to 81 per cent. In those plants in which the percentage aborted pollen was greater than 30 per cent collapsed microspores were first observed interspersed among plump microspores in the anthers. The course of development of the microspores was compared, therefore, in pollen fertile and pollen sterile hybrids.

During the separation of the microspores from their tetrads, in anthers of the pollen fertile hybrid H-64, the tapetal tissue was composed of comparatively short, thick cells with large nuclei which stained heavily. The locular fluid in which the microspores were suspended was stained readily at this stage. While the microspores became round, enlarged, and more deeply stained, the tapetal cells and their nuclei elongated and conformed with the increasing inner circumference of the locule. As development of the microspores proceeded the nuclei of the microspores migrated to the perimeter of their respective cells where a mitotic division led to binucleate pollen grains. The tapetal cells continued to elongate and adhere to the parietal layer as shown in Figure 3 A, and appeared to remain functional. As the pollen grains near maturation the tapetal tissue became absorbed and finally ceased to exist in the anthers (Figure 3 C).

Behavior of the tapetum in the highly pollen sterile hybrids stands in marked contrast to that just outlined for the pollen fertile hybrid H-64. The differences may be summarized as follows: (1) Elongation and adherence of the tapetal tissue to the adjacent tissue failed to occur in differing degrees as the microspores began their first phase of growth; (2) Coincident with this phenomenon a number of the developing microspores became vacuolate and shrunken as might be expected in the case of a nutrient deficiency; (3) In some of the tapetal cells the usual elongation was observed and the development of some plump pollen followed in consequence.

In anthers of the pollen sterile hybrids, during the separation of the microspores from their tetrads, the tapetal tissue stained heavily and short thick cells with large nuclei were observed. At this time the locular fluid was noticeably stained. As the microspores began to round up and enlarge the tapetal cells remained short and thick, and later lost contact with the adjacent tissue (Figure 3 B). During the tapetal behavior just outlined and subsequently a high percentage of the microspores became vacuolate and shrunken until finally the microspores appeared as empty cells. Even though this characteristic abnormal behavior occurred in a high percentage of the tapetal cells some cells behaved in a normal manner. The condition was noted more readily in the longitudinal sections of the anthers. The locules were shrunken with only a few plump pollen grains occurring in each (Figure 3 D).

Inheritance Studies

The plants characterized by anther dehiscence showed a wide range of pollen sterility in both F_1 and F_2 generations. The distribution of the percentage aborted pollen in 100 F_1 plants analysed under greenhouse and field conditions is shown in Figure 4. Most of the variates were intermediate in percentage aborted pollen with, however, a small group at the extremes of the distribution. The mean percentage aborted pollen of series A and B was 50.5 and series C 40.9 per cent, respectively. This represents a reduction of 10 per cent mean aborted pollen when the plants were grown and analysed under field conditions in contrast with less favourable environmental conditions under greenhouse culture.

A summary of all the data on aborted pollen in the F_1, F_2 and S_1 families is shown in Table 2. It can be observed that considerable dispersion occurs in all families with the exception of the selfed progeny of the male fertile parent 50–46 and the selfed progeny of the hybrid H-13.

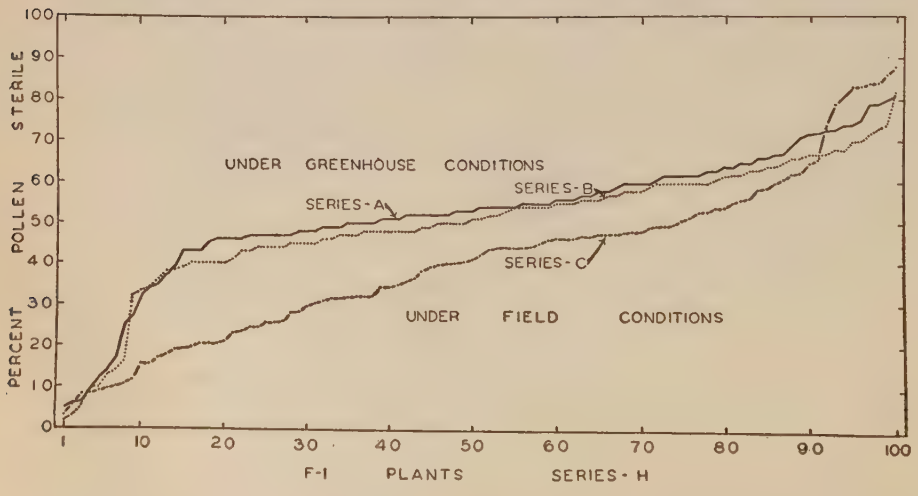


FIGURE 4. The percentage of pollen sterility in 100 F_1 plants. These data were obtained under greenhouse and field conditions and were arranged in ascending order of percentage pollen sterility.

TABLE 2.—FREQUENCY TABLE OF THE PERCENTAGE ABORTED POLLEN IN FIELD GROWN F_1, F_2 AND S_1 PLANTS

Parent Plant No.	Generation	Per Cent Abortion of Parent	Mid-points of Classes																		Total Plants	Mean Per cent Aborted
			3	8	13	18	23	28	33	38	43	48	53	58	63	68	73	78	83	88		
F_1-1	F_1	—	1	6	2	7	7	7	11	7	10	16	6	6	4	1	1	2	4	2	100	40.9
F_1-2	F_1	—	1	1	1	1	2	3	5	5	11	7	3	2	1	2	1	0	1	2	49	43.3
H-8	F_2	20	3	6	22	20	17	15	18	11	12	5	7	3	1	3					143	28.1
H-57	F_2	30	4	5	3	3	3	4	3	3	4	3	3	1	0	2	0	1			42	35.1
H-71	F_2	41		1	5	3	7	8	4	10	7	7	2	1	0	2					65	36.5
H-46	F_2	42				3	6	10	8	10	16	24	8	11	4	3					103	42.4
H-74	F_2	44				3	6	11	10	14	15	10	3	6	2	5	0	1			86	41.4
H-35	F_2	55				1	0	2	4	8	10	8	15	9	10	5	2	2	2		78	52.0
H-3	F_2	71						1	3	0	2	1	2	2	4	2	2	3			22	56.4
H-13	F_2	5	4	44	48	48	36	22	18	5	5	2									232	18.9
50-46	S_1	9		2	7	9	9	13	3	5											48	22.9

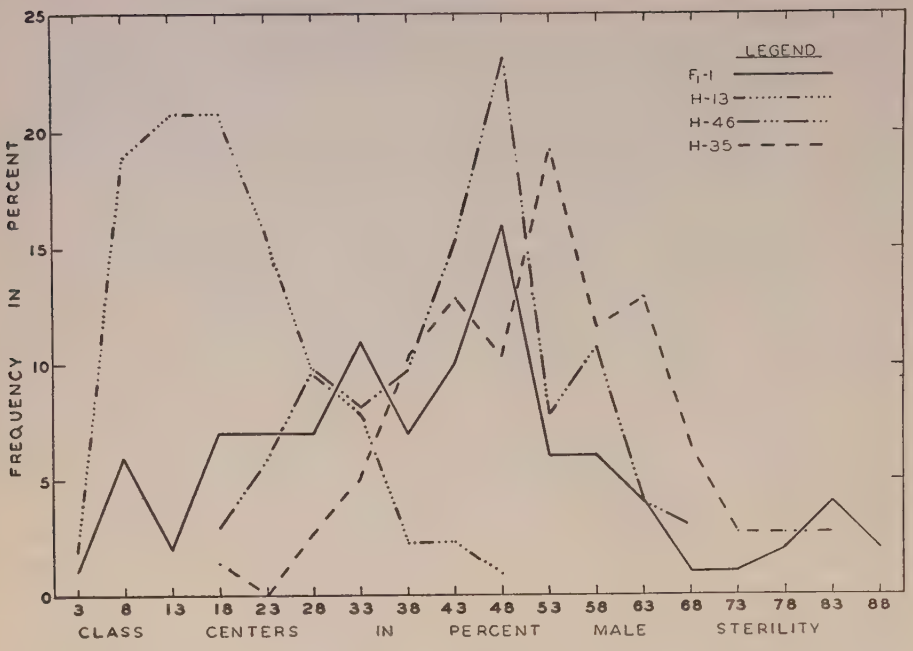


FIGURE 5. Graphic presentation of the distribution of per cent pollen sterility in F_1 and three F_2 families.

A graphic presentation of the distribution of male sterile variates in four families is shown in Figure 5. The F_1 data from series C were plotted and also progeny from the hybrids H-13, H-46 and H-35 which had 5.5, 41.0 and 54.0 per cent aborted pollen, respectively. The number of variates and the progeny means of each family are shown in Table 2.

The distribution of pollen sterility in the F_1 generation shown by the unbroken line exhibits the greatest dispersion. Two small peaks are evident at the extremes of the distribution viz., 8 and 83. The balance of the population is distributed into two groups, between 13 and 38, and between 38 and 64. The low point at the class centre 38 lies only two points left of the arithmetic mean of the population.

The selfed progeny from H-13, a variate from the extreme left class of the F_1 population, fall predominantly into three classes represented by the class centres 8, 13 and 18. The selfed progeny from H-46, a variate secured from approximately the mean of the F_1 distribution, fall into three groups with peaks at 28, 48 and 58 per cent respectively. The selfed progeny from H-35, a variate selected from the right of the mean of the F_1 distribution, fall roughly into five groups: three peaks at 43, 53 and 63 with an additional group at each end of the distribution.

A test was made to determine whether a small number of genes having a non-dominant cumulative effect on the presence of aborted pollen were acting in this material. The male fertile parent 50–46 was considered as homozygous recessive (neutral) for the male sterile genes. Due to the recurrence of parental types in the F_1 generation, the male sterile parent was considered to have either one, two, three or four genes in the heterozygous condition. The theoretical distribution of the expression of partial

TABLE 3.—SUMMARY OF CHI-SQUARE DATA TESTING ALTERNATE RATIOS IN F_1 AND F_2 FAMILIES WHEN THE PER CENT ABORTED POLLEN DATA WERE TRANSFORMED TO DEGREES OF $\sin^2\phi$.

Family	No. of plants	1 : 2 : 1		1 : 3 : 3 : 1		1 : 4 : 6 : 4 : 1	
		χ^2	P	χ^2	P	χ^2	P
F_1	100	6.08	.03	1.12	.77	6.71	.16
H–46	103	1.24	.54			8.07	.09
H–74	86	7.58	.02			29.86	>.01
H–71	65	.50	.78			7.46	.12
H–57	42	2.72	.26			20.07	>.01
H–8	143	15.42	>.01			17.78	>.01
H–35	78	6.38	.04			1.33	.93

male sterility under each of the four conditions was considered with the number of classes involved in each hypothetical case. The actual distribution of the variates in the F_1 population was tested by partitioning the total range of variability obtained into equal classes, according to the number of genes presumed to enter from the male sterile parent. The partitioning of the total variability was accomplished by calculating the class interval. This was followed by a calculation of the chi-square test for goodness of fit. The resulting chi-square values were required to estimate the most probable genetic interpretation based on the distribution of the percentage of aborted pollen in the F_1 progeny. The same method was used for the analysis of the F_2 data. Percentage pollen abortion data were converted into degrees of $\sin^2\phi$ before analysis.

A summary of the chi-square data is shown in Table 3. The F_1 generation chi-square values are shown for a 1 : 2 : 1, 1 : 3 : 3 : 1 and a 1 : 4 : 6 : 4 : 1 ratio. The lowest chi-square value and the highest P value correspond with a 1 : 3 : 3 : 1 ratio.

The chi-square values in the F_2 progeny of H-46, H-71 and H-57, as shown in Table 3 indicate a good fit to a 1 : 2 : 1 ratio for the presence of aborted pollen in the population. A significant departure from a 1 : 2 : 1 ratio was obtained when the progeny of H-74 was tested. However, a larger chi-square value was obtained for the alternate 1 : 4 : 6 : 4 : 1 ratio. In H-8 there was a significant departure from 1 : 2 : 1 ratio. The means for percentage aborted pollen in the above five families were all below 43 per cent.

The chi-square value in the F_2 progeny of H-35, which had a mean of 52 per cent pollen abortion, indicates a good fit to a 1 : 4 : 6 : 4 : 1 ratio.

Fertility Studies

The 100 F_1 plants were selfed in order to produce F_2 seed. The number of florets selfed varied in different plants. In 69 per cent of the plants from 100–300 florets were selfed, in 15 per cent less than 100 and in 16 per cent over 300. The previous genetic results indicated a good fit to a 1 : 3 : 3 : 1 ratio for partial pollen sterility in the F_1. This suggested that the lower 1/8 or 12.5 per cent of the population would not contain the factors which were considered to be responsible for the occurrence of pollen abortion in the remaining 87.5 per cent of the F_1. Therefore, only 87 plants, which had the highest percentage aborted pollen, were included in the correlation study.

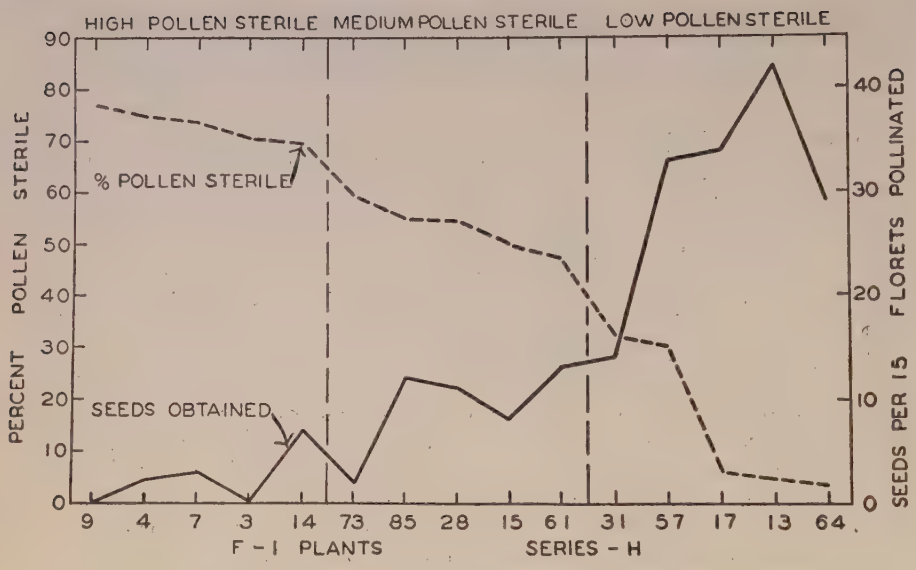


FIGURE 6. Graphic representation of pollen sterility in selected F_1 plants in three pollen sterility groups and the seeds obtained when 15 florets of (P–1–3) were pollinated by the above F_1 plants.

The mean per cent aborted pollen (Series A and B) data were transformed to degrees of $\sin^2\phi$. Then the correlation between the percentage aborted pollen and number of seeds per floret selfed was calculated. The resulting coefficient $r = -.575$ was highly significant. Such a negative association indicated that the presence of aborted pollen had a depressive effect upon self-fertility.

A test was made to determine the effectiveness of the pollen parents which varied considerably in percentage pollen sterility. Therefore, three pollen sterility groups, from 3 to 32 per cent, from 47 to 60 per cent and from 70 to 78 per cent, were established with five plants in each group. The completely male sterile plant P-1-3 was used as the female parent in this experiment. Pollen was collected on a toothpick from each F_1 plant and applied to 15 florets of the female parent. Care was taken to provide pollen from 6 to 8 florets for each 4 to 6 florets pollinated. Pollination was completed during a 48 hour period for all F_1 plants.

Data are presented graphically (Figure 6). It can be readily observed that the trend in the number of seeds obtained per 15 florets pollinated varies inversely with the percentage of pollen abortion in the pollen parents. It appears that the number of viable pollen grains deposited on the stigma were mainly responsible for this result but there may be other contributing factors.

The same grouping of F_1 plants was maintained in a study of comparative female fertility, in which four units of 15 florets of each plant were pollinated by four unrelated male fertile plants, thus giving a quadruple test of female fertility for each plant. A graphic presentation of the data in Figure 7 shows considerable variation in seed set. But in contrast to

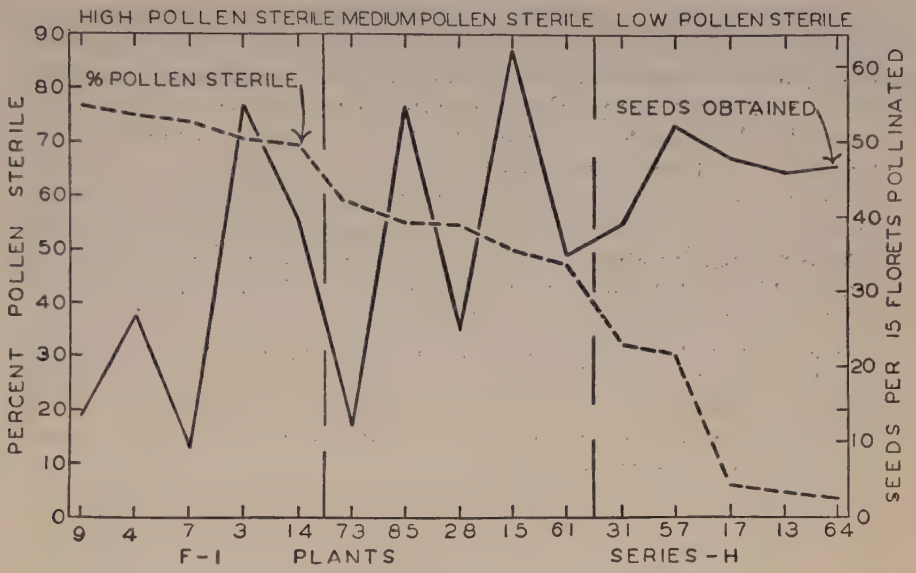


FIGURE 7. Graphic comparison of female-fertility in F_1 plants in three pollen sterility groups as measured by the pollination of 15 florets of the F_1 plants by each of 4 unrelated pollen fertile plants.

the results presented in Figure 6 there does not appear to be a direct relationship between percentage pollen sterility and seed set when these F_1 plants were tested as females. Furthermore, these data suggest that male and female sterility are independent phenomena.

DISCUSSION

Complete male sterility was characterized by a precocious development and overgrowth by the tapetal tissue which was associated with the vacuolation of the sporogenous cells leading to the degeneration of the pollen mother cells in the early prophase stage. Partial male sterility was characterized by a swollen tapetum. This condition was observed previous to the first mitotic division of the microspores. Some of the microspores fail to grow and those spores affected become vaculolate and shrunken. Normal appearing pollen grains occurring at random among the shrunken grains were viable in self-fertilization studies.

The inheritance of non-dehiscence was attributed to the action of duplicate genes*. The symbols Ms_1 , and Ms_2 were used to refer to these male sterile genes. The cross and the resulting F_1 and F_2 generations could be expressed as follows:

	P–1–3		50–46
P_1	$ms_1\ ms_1\ ms_2\ ms_2$	×	$Ms_1\ Ms_1\ Ms_2\ ms_2$
F_1	$Ms_1\ ms_1\ ms_2\ ms_2$	and	$Ms_1\ ms_1\ Ms_2\ ms_2$
F_2	3 : 1		15 : 1

* Since the preparation of this paper, Stanford (Agron. J. 43 : 222–225. 1951) has established the occurrence of tetrasomic inheritance for purple flower colour in alfalfa. As F_2 populations were not included in the present study, no attempt could be made to determine whether tetrasomic inheritance was operative in the factors determining male sterility.

The inheritance of partial male sterility is not a simple phenomenon. It involves quantitative characters whose expression is influenced by sampling and environmental effects, which tend to spread the pollen sterility values continuously rather than to form discrete classes or groups. An attempt has been made to explain the differences between families within the limits of the present investigation.

The fact that a great majority of the F_1 variates fall in an intermediate position relative to the parents implies the action of quantitative factors on the percentage of aborted pollen. In general the distribution was continuous. Contrary to most distributions of variable data there was a low point in the distribution at the arithmetic mean 40.9.

The working hypothesis, that the male sterile parent was heterozygous for major partial male sterile genes, was tested. The lowest chi-square value and the highest P value corresponded to a 1 : 3 : 3 : 1 ratio. On the basis of this test alone the following combinations of gametes were believed to account in part for the F_1 results:

P–1–3 × 50–46

Female gametes

	3 Doses	2 Doses	1 Dose	0 Dose
	$A_1A_2A_3$	$A_1A_2a_3$ $A_1a_2A_3$ $a_1A_2A_3$	$A_1a_2a_3$ $a_1A_2a_3$ $a_1a_2A_3$	$a_1a_2a_3$
Male gametes	$a_1a_2a_3$			
F_1 ratio	1	3	3	1

The mean of the F_2 progeny of H-13 (5.5% aborted pollen) was 18.9. This was 4 per cent lower than the mean of the S_1 generation of the male fertile parent 50–46. On the basis of this comparison, it was considered that there were no major genes for partial male sterility in the hybrid H-13.

The progenies of H-46, H-71 and H-57 corresponded with populations in which segregation occurred in accordance with a 1 : 2 : 1 ratio on the basis of chi-square tests. On the basis of these data the above three plants were each considered to be heterozygous for one major gene for partial male sterility which could be represented by (Aa).

The progeny mean of H-74 was only 1 per cent lower than the progeny mean of H-46. However, the chi-square test showed a significant departure from a 1 : 2 : 1 ratio in H-74. It was observed that the two distributions follow a similar course until the higher pollen sterility mode was reached. There appeared to be fewer individuals in the progeny of H-74 in this high sterility group. Where the parents are highly heterogenous, as in the present case, it is impossible to explain this deviation.

The progeny mean of H-8 was 28.1 per cent. The chi-square test indicated a significant departure from a 1:2:1 ratio. Approximately 25 per cent of the population of the F_2 progeny was completely male sterile.

The progeny mean of H-35 was 52 per cent. The chi-square value for a 1 : 4 : 6 : 4 : 1 ratio was 1.33 with a P value of .93; whereas for a 1 : 2 : 1 ratio the chi-square value was 6.38 with a P value of .04. On the basis of these data the most probable interpretation of the inheritance of partial pollen sterility in this family would appear to involve the random segregation of two similar gene pairs.

No attempt was made to interpret the F_1 data, except in a general way, before the F_2 families were planted out and the pollen sterility data obtained. Therefore it was not possible to plan the experiment or form any preconceived hypothesis before the completion of the collection of all the pollen sterility data. On the basis of the F_1 and F_2 data analysed the parental genotypes relative to partial pollen sterility are tentatively constituted as follows:

Male fertile parent			*Male sterile parent*		
a_1a_1	a_2a_2	a_3a_3	A_1a_1	A_2a_2	A_3a_3

A high negative correlation between self-fertility and male sterility was established in this experiment. The practice of selecting for high self-sterility in alfalfa previous to polycross testing has been followed by some plant breeders. This experiment suggests the importance of analysing for male sterility to insure that it is not the causal agent in self-sterility. Furthermore, it shows also that partially pollen sterile plants produced considerably less seed than pollen fertile plants under the same conditions. Such a result would indicate that the presence of a high percentage of partially male sterile variates in a population might have a detrimental effect on seed-setting.

SUMMARY

1. Complete male sterility is associated with the parallel presence of a swollen tapetum and a complete degeneration of the pollen mother cells during the early prophase.

2. Atypical tapetal behavior at a stage when the young microspores normally undergo rapid growth is considered to be associated with the immediate cause of partial male sterility.

3. A duplicate factor mechanism for the inheritance of complete male sterility is suggested.

4. The inheritance of partial male sterility is attributed to three genes, A_1, A_2 and A_3 segregating at random, which are acting in a quantitative way to give a partially lethal effect.

5. A significant negative correlation of $-.575$ is obtained between pollen sterility and seeds per floret selfed. Furthermore, plants containing a high percentage of aborted pollen are relatively ineffective as pollen parents.

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THE QUANTITY AND QUALITY OF MATURE EWES' MILK AS INFLUENCED BY LEVEL OF PROTEIN IN THE RATION[1]

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In a previous paper from this Station (22) data were presented which indicated that the birth weights and the growth rates of lambs from birth to 6 weeks of age were influenced significantly by the amount of protein consumed by the pregnant and lactating ewe. It was not known whether this increased rate of growth was due to an increased milk supply of the ewes on the higher levels of protein intake or to a higher content of protein or other constituents in the milk produced. Therefore, milk production studies were initiated to determine the effect of the protein intake of lactating ewes upon their milk production and the growth of their lambs.

Neidig and Iddings (13) concluded, from studies involving 3 ewes from each of 6 breeds of sheep, during the period from the tenth to the fiftieth day after lambing, that the quantity of milk produced by the ewe was a more important factor influencing growth rate of the lamb than was the quality of milk produced (amounts of protein, fat, lactose, and ash). The quantity of milk produced was determined by weighing the lamb before and after each nursing during one 24-hour period at intervals of 10 days.

Snell (23) reported that the amount of milk produced by the ewe was influenced by the amount of feed fed during lactation, and that the growth rate of the lamb to 120 days of age was closely correlated with the amount of milk produced by its mother.

Shrewsbury and co-workers (19, 20) found that milk production of ewes was influenced markedly by different roughages. These workers did not feel that the differences in protein content of the various roughages entirely accounted for differences in milk production, since a low-protein ration supplemented with cottonseed cake or casein did not give as good results as when supplemented with alfalfa. They also found that there was a high correlation ($r = +0.87$) between milk production of the ewe and the gain of lamb to 8 weeks of age. In these studies milk production was determined by weighing the lamb before and after nursing at 3-hour intervals during one 24-hour period (12-hour period in their second study) once each week during the first 8 weeks of lactation.

Barnicoat and co-workers (1, 2) reported that, in experiments with Romney ewes, milk yields were not significantly influenced by the live weight of the ewe, but were influenced by the age of ewe, the number of lambs nursing the ewe, and the level of nutrition of the ewe. They reported that level of nutrition during lactation was of more importance in influencing milk yields than level of nutrition during pregnancy but that maximum milk yields were obtained only by liberal feeding during both the latter part of pregnancy and throughout lactation. They found highly significant correlations between the amount of milk produced by the ewe and the growth rate of lambs to 6 weeks of age. This correlation was highest during the period of 4 to 6 weeks after lambing. The correlation between milk production of the ewe and gain to 12 weeks was significant in some years and

[1] Contribution from the Division of Animal Husbandry, Experimental Farms Service, Canada Department of Agriculture, Ottawa.

with some ages of ewes but not significant in all cases. Protein content of the milk from ewes fed a high plane of nutrition during lactation was approximately 10 per cent greater than that from ewes fed a low plane of nutrition (average of 5.65 per cent protein for high plane of nutrition vs. 5.08 per cent for low plane). However, the fat content of the milk from "high plane" ewes was approximately 12 per cent less than that from "low plane" ewes. In these studies milk production data were obtained by weighing the lamb before and after nursing at approximately 4-hour intervals.

Wallace (24) found that the milk production of ewes was influenced significantly by the level of nutrition during the last 6 weeks of pregnancy. He reported that ewes nursing twin lambs produced appreciably more milk than ewes nursing single lambs (47 per cent more for Suffolk ewes and 67 per cent more for Border-Leicester × Cheviot ewes) during a 16-week lactation period. This difference was most pronounced during the first few weeks of lactation. Heavier ewes were reported also to produce more milk than lighter ewes. The growth of the lamb was found to be highly correlated with the milk production of the ewe ($r = +0.92$ at one month of age and $r = +0.71$ at 112 days of age) for Border-Leicester × Cheviot sheep. Each additional pound of milk that the lamb consumed to 28 days of age was found to increase the lamb weight at one month of age by 0.25 pound.

In studies with dairy cows Perkins *et al.* (18) found that the total protein content of the milk from cows receiving a low-protein ration (N.R. = 1 : 13) was approximately 6 per cent less than that from cows receiving a high protein ration (N.R. = 1 : 2). This difference was due largely to an increased amount of non-protein nitrogen in the milk of the cows on the high-protein ration. Bartlett *et al.* (3) reported no difference in the yield or protein content of the milk from cows fed a ration which supplied 0.6 pound of protein equivalent per 10 pounds of milk produced and cows fed 2/3 this amount of protein. Parrish *et al.* (14) found no difference in the protein content of colostrum or early milk from 2 groups of cows when one group received a high-protein concentrate in addition to hay and silage while the other group received a low-protein concentrate plus hay and silage during a period of 7 weeks before and 8 days after parturition. The non-protein nitrogen content of the milk was higher from the group receiving the high-protein ration. Breirem and Ulvesli (5), in Norway, reported that the protein content (N × 6.37) of the milk from cows fed a ration containing optimum quantities of protein was 3.69 per cent whereas that from cows receiving minimum quantities of protein was 3.57 per cent and that from cows receiving a ration deficient in protein was 3.44 per cent. There is general agreement among workers in dairy cattle feeding research that quantity of protein in the rations will influence the amount of milk produced (3, 6, 12, and others).

In practically all experiments on protein requirements of sheep no attempt has been made to maintain energy intake at a constant level and to vary only protein intake. No experiments have been reported, to the authors' knowledge, that were designed to study the influence of protein intake during pregnancy and lactation upon milk production and growth of lambs. Data from such studies would be of value in formulating rations for lactating ewes.

PROCEDURE

Daily milk production over a period of 6 weeks commencing on the seventh day after lambing was obtained from all ewes from each of 3 lots of ewes that had sound udders, that were raising single lambs, and that were consuming their daily ration. Lot 1 received a ration containing 7.3 per cent total protein throughout pregnancy and lactation, Lot 2 received the same ration until 6 weeks before lambing and then a ration containing 10.5 per cent protein thereafter, and Lot 3 received the ration containing 10.5 per cent protein throughout pregnancy and lactation.

The ewes, 3- and 4-year-old grade Corriedales, were fed in individual stalls twice daily. The rations, fed in pelleted form, were composed of 70 per cent grass hay and 30 per cent concentrate (molasses, starch, oats, linseed oilmeal, and minerals). Details regarding the composition of the rations and management of the sheep were similar to those described in a previous paper (21). Digestible crude protein and total digestible nutrient content of the rations were determined by the "lignin-ratio" method. This method, while not as accurate as the "direct" method, is considered to give a reliable index of the digestibility of a ration (7, 11, and others). The lambs had access to supplementary feed in a creep until about 8 weeks of age when pasture was provided for them. However, they took very little supplementary feed until they were about 6 weeks of age.

The ewes were started on their experimental ration in June, 1950, and were continued on these rations until September, 1951, when the lambs were weaned. Milk production for each ewe was determined during a 24-hour period on the 7th, 11th, 14th, 18th, 21st, 25th, 28th, 32nd, 35th, 39th, 42nd, and 46th day after lambing by weighing the lambs before and after each nursing. This method has given more reliable data on milk production of non-milking breeds of sheep than hand milking or the use of hormones (1, 4, 8). The lambs were removed from the ewes at 7 a.m. of the day that milk yields were to be determined and then permitted to nurse at 11 a.m., 3 p.m., 7 p.m., 11 p.m., and at 3 a.m. and 7 a.m. the following morning. Before the 7 a.m. nursing a sample of milk (about 2 ounces) was collected by hand-milking for fat and protein analysis. Fat was determined by the Babcock method and protein ($N \times 6.38$) by the Macro Kjeldahl method using $CuSO_4$ and K_2SO_4 as catalysts.

RESULTS AND DISCUSSION

Table 1 presents the data obtained in this experiment on average daily milk production, protein content of the milk (fat-free basis), fat content of the milk, and growth of the lambs as influenced by the amount of protein received by the lactating ewes.

Milk Production

From the milk production data of the ewes used in this experiment there was a highly significant difference ($P < 0.01$) in daily milk production from ewes receiving approximately 7 per cent total protein in their ration during lactation as compared to those receiving 10 per cent total protein. However there was no difference in the milk production of those ewes that had received 7 per cent protein during early pregnancy and then increased

TABLE 1.—MILK PRODUCTION, PROTEIN CONTENT OF THE MILK, AND GROWTH OF LAMBS AS INFLUENCED BY PROTEIN INTAKE DURING LACTATION

—	Lot 1	Lot 2	Lot 3
No. ewes completing experiment	12	14	14
Av. per cent protein in ration during lactation (D.M. basis)	7.3	10.5	10.5
Av. daily D.M. intake during lactation (lb.)	3.1	3.5	3.5
Av. daily D.C.P. intake during lactation (lb.)	0.11(0.28)[1]	0.25(0.30)	0.25(0.30)
Av. daily T.D.N. intake during lactation (lb.)	1.89(2.7)	2.00(2.8)	2.00(2.8)
Av. weight of ewes at lambing (lb.)	131	140	146
Av. wt. ewes 8 wk. after lambing (lb.)	110	118	125
Loss during first 8 wk. of lactation (lb.)	21	22	21
Av. daily wt. milk produced (oz.)	29.6	42.5*	42.2*
Av. per cent protein in milk (fat-free basis)	4.40	4.66*	4.84*[2]
Av. daily amount protein in milk (oz.)	1.21	1.87*	1.90*
Av. per cent fat in milk	7.9	8.5	8.5
Av. wt. of lambs at birth (lb.)	9.4[3]	10.3[4]	10.7[5]
Av. wt. of lambs at 7 weeks of age (lb.)	31.1	39.5*	39.4*
Av. gain to 7 weeks of age (lb.)	21.7	29.2*	28.7*
Av. weaning wt. (5 mo.) (lb.)	64.0	74.5*	74.5*
Av. gain to weaning (lb.)	54.6	64.2*	63.8*

* Significantly greater than lot 1 ($P < 0.01$).
[1] Numbers in parentheses are the National Research Council's Recommended Allowances (15).
[2] Significantly greater than lot 2 ($P < 0,.01$).
[3] 5 lambs born as twins, raised as singles.
[4] 9 lambs born as twins, raised as singles.
[5] 7 lambs born as twins, raised as singles.

to 10 per cent during the latter part of pregnancy and throughout lactation as compared to those receiving 10 per cent throughout pregnancy and lactation. This would indicate that the amount of protein in the ration during the early part of pregnancy does not influence significantly the milk production of the ewe.

The average daily milk production obtained in this study for the 2 groups fed the higher level of protein was very similar to that reported by Barnicoat *et al.* (1) for New Zealand Romney ewes fed a high plane of nutrition, somewhat lower than that reported by Neidig and Iddings (13) for the average of 6 mutton breeds of sheep, and by Wallace (24) for Suffolk and Border-Leicester × Cheviot ewes, but much higher than that reported by Snell (23) for Cheviot ewes, by Bonsma (4) for Merino and Merino cross-bred ewes, by Peirce (16, 17) for Merino ewes, and by Shrewsbury *et al.* (19, 20) (breed not stated). Wallace (24) and Barnicoat *et al.* (1) reported that ewes nursing twins produced considerably more milk than those nursing singles. Only singles were used in the present experiments since none of the ewes in the low-protein group seemed capable of raising twin lambs. The weaker lamb in all sets of twins in Lot 1 was destroyed soon after birth.

The ewes in the low-protein group were somewhat lighter in weight during lactation than those in the other groups, but this should not have influenced greatly the milk production of these ewes. The average weights of the 3 groups of ewes were approximately the same when placed upon the experimental rations. Barnicoat *et al.* (1) found no significant correlation between body weight of ewe and milk production, when the difference

TABLE 2.—THE INFLUENCE OF DAYS AFTER PARTURITION UPON MILK YIELD AND PROTEIN CONTENT OF THE MILK

Days after parturition	Av. daily milk yield (oz. per day)			Av. protein content (per cent fat-free basis)		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 5
7	43	50	50	4.95	5.20	4.98
11	39	48	48	4.46	4.41	4.84
14	35	45	47	4.16	4.57	4.84
18	32	46	47	4.12	4.50	4.77
21	29	44	44	4.28	4.38	4.64
25	30	46	45	4.13	4.46	4.64
28	27	41	45	4.17	4.57	4.70
32	27	38	40	4.35	4.63	4.75
35	28	39	39	4.40	4.75	4.84
39	23	39	37	4.48	4.74	4.99
42	22	36	35	4.70	4.88	5.04
46	22	36	31	4.55	4.87	5.04
Average	29.6	42.5	42.2	4.40	4.66	4.84

in body weight was mainly due to differences in fatness of the ewe. However, Bonsma (4) and Wallace (24) reported that heavier ewes produced more milk than lighter ewes. There were no significant differences in weight losses of the ewes during the first 8 weeks of lactation indicating that all groups were drawing on body stores of nutrients to about the same extent. The ewes in Lot 1 were receiving about 5 per cent less total digestible nutrients than those in the other two groups. This may account for some of the difference in milk yield between Lot 1 and Lots 2 and 3, although the linear regression coefficient for milk production of these ewes on their individual total digestible nutrient intake was non-significant.

Table 2 shows the average daily milk yields and protein content of the milk as influenced by the number of days after lambing. These data are presented graphically in Figure 1. It is evident from these data that the ewes had reached their maximum production during the first week of lactation and then gradually declined in production. Lot 1 declined in production at a faster rate than Lots 2 and 3. Wallace (24), Barnicoat *et al.* (1), and others have reported that the maximum production of well-fed ewes nursing single lambs was reached during the second to third week of lactation. These workers attributed this in part to the inability of the lamb to consume all of the milk that the ewe was capable of producing during the first 2 weeks. However, a number of the lactation curves presented by Barnicoat *et al.* (1) are similar to those presented in this paper.

Protein Content of Milk

The protein content of the milk is presented on a fat-free basis because of the variability of fat content of samples collected when the udder is not completely milked out.

The protein content of the milk (fat-free basis) from the ewes receiving the low-protein ration was significantly less ($P < 0.01$) than that from the other 2 groups. The percentage of protein in the milk from the ewes receiving 10 per cent protein throughout pregnancy and lactation was

significantly greater ($P < 0.01$) than that from ewes receiving 7 per cent during early pregnancy and 10 per cent during the latter stages of pregnancy and during lactation. Apparently, the latter group of ewes did not have as great a store of body protein upon which to draw as the group fed the higher level of protein throughout pregnancy and lactation. Since non-protein nitrogen was not determined in these samples it is not known whether the increase in protein ($N \times 6.38$) was an increase in "true" protein or an increase in non-protein nitrogen as reported by Perkins *et al.* (18) with dairy cows. The percentages of total protein ($N \times 6.38$) in the milk of mature ewes reported in this paper are similar to those reported by Peirce (16, 17) but are lower than those reported by Barnicoat *et al.* (2), Godden and Puddy (9), and Bonsma (4).

From the data reported in Table 2 and Figure 1 it will be seen that the protein content of the milk was highest during the first week of lactation, gradually declined during the following 2 weeks, and then increased again during the remainder of the period under study. Peirce (16, 17) found a steady increase in the total protein content of the milk of ewes from the second to ninth week of lactation. The increase was from approximately 4.3 per cent during the second week to approximately 5.7 per cent during the ninth week of lactation. Godden and Puddy (9) observed a similar trend.

Fat Content of Milk

The fat content of the milk as influenced by protein intake also is shown in Table 1. Since the udder was not milked out completely in collecting the sample of milk for analyses the fat content data are subject to considerable error, and too much significance cannot be attached to these data. However, the data indicated that the fat content of the milk increased with the protein content. This does not agree with the findings of Barnicoat *et al.* (2) whose data show that there was an inverse relationship between protein content and fat content. The average fat values found in this experiment were slightly lower than those reported by Neidig and Iddings (13), Peirce (16, 17), and Godden and Puddy (9), but higher than those reported by Barnicoat *et al.* (2) and Bonsma (4).

Lamb Growth

The lambs nursing the ewes in this experiment from Lots 2 and 3 made significantly ($P < 0.01$) faster gains to 7 weeks of age and to weaning time (5 months) than those in Lot 1. There was no significant difference between the gains of the lambs in Lots 2 and 3.

In this experiment none of the groups received the National Research Council's recommended allowances for protein (15). However, the lambs and ewes in Lots 2 and 3 made very satisfactory gains indicating that 0.25 pound of digestible crude protein daily during lactation was a satisfactory allowance for a range ewe weighing approximately 140 pounds at lambing and raising a single lamb. The ewes in these experiments received approximately two-thirds of the National Research Council's recommended allowance for total digestible nutrients (15) as it was not possible to induce them to consume more than this amount.

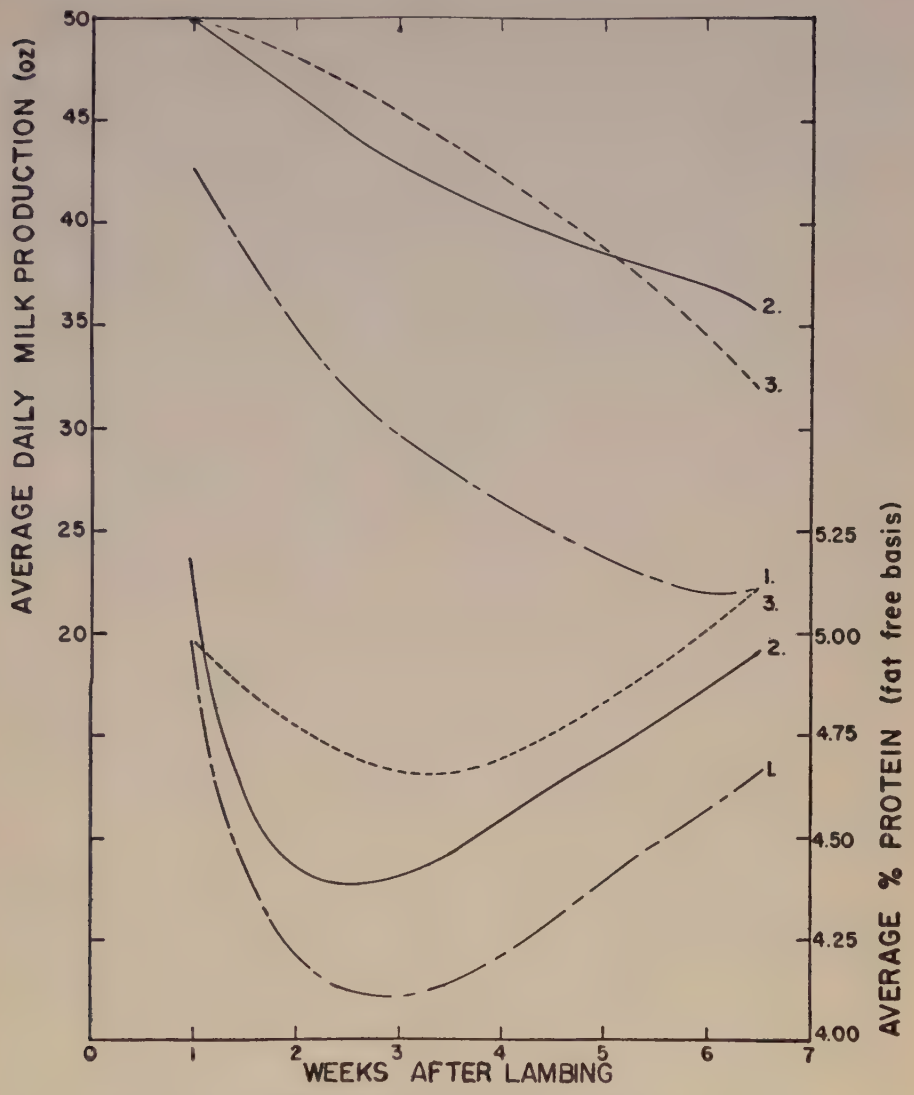

FIGURE 1. Average daily milk production (oz.) and average per cent protein (on fat-free basis) in the milk as influenced by level of protein in the ration and stage of lactation. 1, 2, and 3 are Lots 1, 2, and 3 respectively.

Correlation between Milk Produciton and Lamb Growth

There was a highly significant correlation ($r = +0.76$) between the gains of the lambs from birth to 7 weeks of age and the average daily milk production of their mothers. There was also a highly significant correlation ($r = +0.81$) between the gain of the lambs to 7 weeks of age and the amount of protein secreted in the milk of their mothers. However, the correlation between the gain of the lambs to 7 weeks of age and the percentage protein (fat-free basis) in the milk of their mothers was not significant. This indicates that the high correlation between lamb gains and the amount of protein secreted in the milk of their mothers was due largely to the

increased amount of milk secreted rather than to a higher percentage of protein in the milk. Neidig and Iddings (13) concluded from their study that the quantity of milk produced by the ewe was a more important factor influencing the growth rate of the lamb than was the quality of milk produced.

There were also highly significant correlations between the gain of the lamb to weaning time (5 months of age) and the average daily milk production of its mother during the first 7 weeks of lactation ($r = +0.63$) and between the weight of lamb at weaning and average daily milk production of its mother ($r = +0.65$). However, there was no significant correlation between average daily milk production of the ewe during the first 7 weeks of lactation and the gain of her lamb from 7 weeks of age to weaning time. Barnicoat (2) and Wallace (24) also reported that as the lamb grew older milk production of the ewe had less influence on the gains made by the lamb.

The correlation between weight of lamb at 7 weeks and at weaning time was highly significant ($r = +0.70$) indicating that even though the lamb did not continue to gain after 7 weeks in proportion to its gain during the first 7 weeks, lambs that were heavier at 7 weeks were also heavier at 5 months (weaning time). Hammond (10) reported a very high correlation between weight of ewe lambs at 5 weeks of age and 20 weeks of age.

The linear regression coefficients for lamb gain to 7 weeks of age and to weaning, as influenced by milk production of the ewe showed that for each ounce increase in the average daily milk production of the ewe between 7 and 46 days after parturition, the lamb gained 0.48 pound more to 7 weeks of age and 0.63 pound more to 5 months of age. The former agrees closely with the findings of Wallace (24) who reported an increase of 0.25 pound in body weight of the lamb at one month of age for each additional pound of milk consumed by the lamb to 28 days of age.

The highly significant correlation and regression coefficients between milk production and lamb growth to 7 weeks and to 5 months of age indicate that lamb growth could be used as a reliable index of the quantity of milk produced by a ewe. This was pointed out also by Shrewsbury *et al.* (19, 20), by Wallace (24), and by Barnicoat *et al.* (2). Since the lamb is almost entirely dependent upon its mother's milk as a source of nutrients during the first 4 to 6 weeks of its life, lamb growth during this period should be a more reliable index of milk production of the ewe than the growth to weaning time as quantity and quality of pasture and supplementary feeds would influence weaning weights to some extent.

Protein and Fat Content of the Colostrum

The average protein and fat content of the first colostrum from the ewes sampled were 19.4 per cent (fat-free basis) and 13.8 per cent, respectively. Differences in protein intake during pregnancy had no significant influence on the protein content or the fat content of the first colostrum. There was a wide variation in the protein and fat content of samples taken from ewes within the same group. Parrish *et al.* (14) reported no difference in the protein content of cow colostrum from feeding different levels of protein during pregnancy and early lactation.

SUMMARY

Milk production studies were conducted with 3- and 4-year-old range ewes from the 1st to 7th week of lactation, by weighing the lambs before and after nursing during one 24-hour period twice weekly. The rations, individually fed, provided 0.11, 0.11 raised to 0.25 six weeks before lambing, and 0.25 pound daily of digestible crude protein during pregnancy and lactation. The total digestible nutrient content of all rations was essentially the same. From the data reported the following conclusions seem justified:

1. Milk production was influenced significantly by the protein intake of the ewes during pregnancy and lactation.

2. The percentage of protein (fat-free basis) in the milk also was influenced significantly by the protein intake of the ewe during pregnancy and lactation.

3. The growth rate of the lamb from birth to 7 weeks of age and to weaning time was highly correlated with the amount of milk produced by the ewe. Growth rate of the lambs to 7 weeks of age was a reliable index of the quantity of the milk produced by the ewe. For each increase of one ounce of milk produced daily by the ewe during the first 7 weeks of lactation the lamb gained 0.48 pound more to 7 weeks of age.

4. The protein requirement for lactation closely approximates 0.25 pound of digestible crude protein per head daily for a 140-pound ewe raising a single lamb.

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The authors wish to acknowledge the assistance of J. Orr, Herdsman, in conducting these experiments.

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FURTHER OBSERVATIONS ON THE EFFECT OF LEVEL OF PROTEIN IN THE RATION OF THE MATURE EWE ON WOOL AND LAMB PRODUCTION[1]

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Recent studies with mature range ewes at this Station (1, 2) have indicated that a ration containing approximately 7 per cent protein (0.13 pound of D.C.P. daily) was adequate for body weight maintenance until the latter stages of pregnancy but was not adequate for maximum wool and lamb production. Rations containing 10 or 13 per cent protein (0.23 and 0.29 pound of D.C.P. daily) were adequate for satisfactory wool and lamb production. However, feeding the higher level of protein (i.e. 13 per cent) did not result in a greater production of clean wool or heavier lambs at birth, 6 weeks of age, or at weaning than was obtained with the ration containing 10 per cent protein. Since the 7 per cent protein ration supplied sufficient protein for normal body gains until the latter part of pregnancy and since other workers (3, 4, 5, 6, and others) have shown that it is during the latter part of pregnancy and during lactation that the body requirements for most nutrients are the highest, a further experiment was carried out to study the influence on wool and lamb production of increasing the protein content of the ration from approximately 7 per cent to 10 per cent about 6 weeks before lambing. Data from this experiment are presented in this paper.

PROCEDURE

Three groups of 20 mature range ewes similar to those used in the previous experiment were used in the 1950-51 experiment. Lot 1 received a ration containing 7.7 per cent total protein, Lot 2 received the 7.7 per cent protein ration until 6 weeks before lambing (February 14, 1951) and then was changed to a ration containing 10.5 per cent protein, and Lot 3 received the 10.5 per cent protein ration throughout the experiment. The ewes were started on feed immediately after shearing in June, 1950, and were removed from experiment in September, 1951, when the lambs were weaned. Details regarding rations fed, methods of feeding, and handling of ewes were described in a previous paper (1). As in the previous study data were collected on total fleece weights, bi-monthly weights of wool from a measured area, staple lengths, fibre thickness, density of fibres on the skin, birth weights of the lambs, weekly weights to 7 weeks of age, and weaning weights of the lambs.

The average daily feed intakes were 3.6, 3.8, and 3.8 pounds (air-dry basis) for Lots 1, 2, and 3, respectively.

RESULTS AND DISCUSSION

Wool Production

The effects of level of protein in the ration of mature ewes on total wool production are summarized in Table 1. In so far as the data from the 7.7 and 10.5 per cent rations are concerned they confirm those obtained

[1] Contribution from the Division of Animal Husbandry, Experimental Farms Service, Canada Department of Agriculture, Ottawa.

TABLE 1.—THE EFFECT OF VARIOUS LEVELS OF PROTEIN IN THE RATION ON WOOL PRODUCTION

		Lot 1	Lot 2	Lot 3
		7.7 per cent protein	7.7 per cent raised to 10.5 per cent protein[1]	10.5 per cent protein
Average daily D.C.P. intake	(lb.)	0.13	0.13 → 0.22	0.22
Average daily T.D.N. intake	(lb.)	2.0	2.0	2.0
Raw fleece weight	(lb.)	7.8	8.4	9.4**
Clean fleece weight	(lb.)	4.0	4.0	4.9**
Yield clean wool	(%)	51.4	48.5	51.9
Dirt loss in breaker	(%)	26.2	30.8	28.9
Staple length	(mm.)	77.7	82.8	86.8**
Fibre thickness (microns)	1950	25.4	24.8	25.6
Fibre thickness (microns)	1951	24.5	23.8	25.7*
Density of fibres per sq. in.		15,937	16,635	16,192

* Significantly greater than Lot 2 ($P < 0.01$).
** Significantly greater than Lot 1 ($P < 0.01$).
[1] Six weeks before lambing.

in the previous experiment (1). The average weights of raw and clean wool are somewhat less but this may be due to a lower energy intake throughout this experiment as compared to the 1949-50 experiment. It should be noted that, in Lot 2, raising the protein content of the ration from 7.7 per cent to 10.5 per cent 6 weeks before lambing increased raw wool production slightly. However, since there was no increase in clean fleece weight this may be attributed to more impurities in the wool. The length and thickness of fibres from the ewes in Lot 3 also were significantly greater ($P < 0.01$) than from those in Lots 1 and 2. It should be pointed out that the average fibre thickness from Lot 2 was somewhat, though not significantly, lower than from Lots 1 and 3 at the beginning of the experiment and this may account for the lack of increase in clean weight from Lot 2 since on the basis of the bi-monthly wool production (see Table 2) a small increase should have been obtained.

The bi-monthly weights of clean wool from a measured area are shown in Table 2. The data from Lots 1 and 3 are very similar to those obtained on the same rations during 1949-50. There were significant ($P < 0.01$) increases in weights of clean wool from June until December, 1950. During the period December 10, 1950 to February 7, 1951, a significant decline ($P < 0.05$) occurred. In the previous paper (1) this decline was attributed mainly to very cold weather (— 11° F. for 40 days) and possibly to early pregnancy. In view of the mild weather that prevailed during this period in the present study the decrease must be attributed to early pregnancy. As in the previous study advanced pregnancy and early lactation significantly ($P < 0.01$) reduced the weight of clean wool grown.

It should be noted that the periodic weights of clean wool from Lot 2 were somewhat, though not significantly, lower than Lot 1 until the end of the fourth period, although both groups received the same ration. As indicated above this was probably due to the fact the ewes in Lot 2 were

TABLE 2.—THE BI-MONTHLY WEIGHTS OF CLEAN WOOL FROM THE TATTOOED SHOULDER AREAS (4 SQ. CM.)

Period	Average weight of clean wool (mg.)			
	Lot 1	Lot 2	Lot 3	Period means
June 12—Aug. 11, 1950	95.5	89.9	104.3	96.8
Aug. 12—Oct. 10, 1950	121.8	113.9	165.4	139.2
Oct. 11—Dec. 9, 1950	162.6	152.0	195.2	175.4
Dec. 10—Feb. 7, 1951	149.1	139.0	175.6	159.0
Feb. 8—April 8, 1951	83.5	94.4	105.8	95.6
April 9—June 7, 1951	41.9	60.8	70.0	58.5
Lot Means—				
Average weight of clean wool	109.1	108.1	136.0	—
Average fibre thickness (microns)	22.3	21.9	23.7**	—
Average fibre length (mm.)	18.6	19.4*	20.8**	—

Minimum significant differences for weights of clean wool

	5 per cent[1]	1 per cent[1]
Between lot means	11.9	16.4
Between period means	14.5	20.1

* Significantly greater than Lot 1 ($P < 0.05$).
** Significantly greater than Lots 1 and 2 ($P < 0.01$).
[1] Levels of probability.

finer-fibred than the remainder. The data on fibre thickness, fibre length, and weight of clean wool were not available at the time of allotment. Consequently the ewes were allotted to the groups on the basis of body weight and raw fleece weight.

During the last two growth periods (i.e. after the ration of Lot 2 was increased from 7.7 to 10.5 per cent protein) the weights of clean wool from Lot 2 were 24 per cent greater than those from Lot 1. However, the wool production of this lot during these two periods did not equal that of Lot 3 which had been on 10 per cent protein continuously.

Average fibre length was found to increase proportionately with each increase in protein fed.

Lamb Production

The number of lambs born and the average birth weights are shown in Table 3. As in the previous study (2) both single and twin lambs from the ewes on the 10.5 per cent protein ration were significantly heavier ($P < 0.01$) than those from the ewes receiving the 7.7 per cent protein ration. The birth weights of the lambs from the ewes in Lot 2 were equivalent to those in Lot 3, indicating that increasing the level of protein from 7.7 per cent to 10.5 per cent 6 weeks before lambing produced as satisfactory results as feeding the ewes a ration containing 10.5 per cent protein for the entire trial.

TABLE 3.—NUMBER OF LAMBS BORN AND AVERAGE WEIGHTS OF LAMBS AS INFLUENCED BY LEVEL OF PROTEIN IN THE RATION OF MATURE EWES

	Lot 1	Lot 2	Lot 3
Number of ewes bred	19	20	20
Number of ewes lambed	19	20	20
Number of lambs born	29	34	31
Number of lambs alive at birth	28	34	31
Number of lambs alive at 6 weeks of age	16	21	22
Average birth weights (lb.)—			
Singles	10.2	12.0	12.1
Twins	7.5	9.2	8.5
All lambs	8.3	9.7	9.4
Weight lamb per ewe lambed	12.7	16.5	15.0
Weight of all lambs at 6 weeks of age	26.7	34.4	32.1
Weight of all lambs at 5 months of age	63.4	72.4	70.1

Lamb survival was much better during this experiment than in the previous one, particularly in Lot 1. This was attributed primarily to the fact that precautions were taken prior to lambing to prevent pregnancy disease. Molasses was fed at the rate of 0.1 pound per head daily in open troughs during the last 5 weeks of pregnancy. As a result no cases of pregnancy disease occurred. In addition the lambs had access to supplementary feed in a creep until about 7 weeks of age when pasture was provided for them. Although the lambs did not consume much of the feed it was felt that it did prevent much of the dirt eating experienced previously.

No sets of twin lambs were raised in the group receiving 7.7 per cent protein because the milk supply of the ewes was inadequate. However, it was found that the ewes whose ration was raised from 7.7 per cent to 10.5 per cent protein produced an amount of milk equal to that from ewes receiving 10.5 per cent protein throughout the trial (7). Average daily milk production for the first 7 weeks of lactation was 29.6 ounces, 42.5 ounces, 42.2 ounces for the ewes in Lots 1, 2, and 3, respectively.

The average weights of lambs to 6 weeks of age and at weaning also are shown in Table 3. The lambs in Lots 2 and 3 were significantly heavier ($P < 0.01$) than those in Lot 1 at birth, 6 weeks of age, and also at weaning. It would appear that feeding the ewes a ration containing 7.7 per cent protein until 6 weeks before lambing had no deleterious effect on lamb gains.

SUMMARY

Three groups of 20 mature range ewes were fed individually, for 15 months, rations containing 7.7 per cent protein, 7.7 per cent until 6 weeks before lambing and then raised to 10.5 per cent protein, and 10.5 per cent protein, to study the influence of protein intake on wool and lamb production.

Average bi-monthly weight of clean wool from ewes receiving the 10.5 per cent protein ration was significantly heavier than those from ewes fed 7.7 per cent protein throughout the experiment or 7.7 per cent to 6 weeks before lambing and then increased to 10.5 per cent.

The bi-monthly weights of clean wool became greater when the protein content of the ewe's ration was increased from 7.7 to 10.5 per cent 6 weeks before lambing as compared to the weights from ewes which remained on the 7.7 per cent ration.

Birth weights of lambs from ewes receiving 7.7 per cent protein until 6 weeks before lambing and then increased to 10.5 per cent were equivalent to those from ewes on 10.5 per cent protein throughout the trial. These weights were significantly ($P < 0.01$) heavier than those from ewes on the 7.7 per cent protein ration. This was true also of the lamb weights at 6 weeks of age and at weaning.

On the basis of the results obtained in this experiment with mature ewes it may be concluded that raising the protein content of the ration from 7.7 to 10.5 per cent 6 weeks before lambing produced as satisfactory lamb production as 10.5 per cent protein fed throughout pregnancy and lactation. However, this was not true for clean wool production.

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PARENT-SEEDLING CHARACTERISTICS AND RELATIONSHIPS IN *CARAGANA ARBORESCENS*, LAM.[1]

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INTRODUCTION

Although an important species for shelterbelt purposes in the plains areas of North America and Russia, *Caragana arborescens*, Lam., (Siberian Pea-tree) has not been subjected to extensive improvement programs.

From studies conducted in the United States (8, 11, 12, 20, 37) and Canada (27, 35) come numerous reports on the value and importance of this species. Over a period of 40 years it has proven the most valuable and versatile tree for general planting in the Canadian Prairies. The Forest Nursery Station at Indian Head, Saskatchewan, annually distributes an average of 1,527,500 seedlings to farmers for field and home shelterbelt purposes. Favorable results in the control of wind erosion by the use of this species have created a demand to improve the species. A characteristic desired from such a program is increased height to assure protection of larger land areas.

In the present study 14 mature trees of *C. arborescens* were selected on the basis of different characteristics. Data were collected from the parental trees on seed-setting by self- and open-pollination to determine their fruitfulness or fertility. Seeds resulting from self- and open-pollination of these trees were studied for seed size and germination amount and speed. The resulting seedlings were then transplanted to the field and studied for vigour. The seed characteristics of parental trees were studied in relation to their seedling progenies, with particular attention being paid to the effects of the characters of the original tree upon the vigour of resulting seedlings. A major purpose of this investigation was to make an intensive study of several factors which might affect the vigour of one-year-old seedlings and to estimate their value in a selection program with *C. arborescens*.

LITERATURE REVIEW

Caragana arborescens, Lam., has been described by Rehder (24) as follows:

> "Deciduous shrub or small tree, growing up to 6 meters of upright habit; leaves pinnate, 8–14 leaflets 1 to 2.5 cm. long, pubescent when young, later glabescent and bright green; flowers yellow, 1 to 4 per fascicle, 1.5 to 2 cm. long, pedicels 1.5 to 2.5 cm. long, articulate; stamens 10 (9 + 1) upper one free; ovary subsessile; style slightly curved; stigma small terminal; ovules 1 to 10; pod linear, 3.5 to 5 cm. long; seed round-oblong; introduced from Manchuria and Siberia in 1752."

Senn (26), following the taxonomical sequence of Faubert, has classified *C. arborescens* in the subfamily *Papilionatae*, tribe *Galegeae* and subtribe *Astragalinae* of Leguminosae. Krueter and Tschechow reported (Senn, 26) a chromosome number of 2n = 16 for this species. Recently Moore (21) has verified their findings and stated "the basic haploid number of the

[1] Portion of a thesis submitted to the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy, December 1950.

[2] Plant Breeder.

genus is 8, and *C. arborescens* is diploid. Meiosis takes place at Ottawa over a period of a week, May 12 to 19; and bivalents are regularly formed." Later Moore reported (22) the average number of ovules per ovary [pod] to be 18.4, and one tree studied exhibited a range of 15 to 21 ovules.

Sexual maturity of *C. arborescens* seedlings, as manifested by their initial blossoming, has been observed by Walker (34) to occur some 4 to 6 years after sowing of the seed.

The following quotations from a 1948 publication of the United States Forest Service (33) summarize published information on *C. arborescens:*

> "The Siberian peashrub is one of the most cold- and drought-resistant deciduous shrubs or small trees in cultivation . . . . flowers appear in May and June and the pods ripen in July, soon splitting open and completely dispersing the seed about mid-August . . . . The seed collection period is seldom longer than 10 days, and pods should be collected just as they begin to open . . . . cleaned seeds range from 13,000 to 22,000 per pound. Russian experience indicates satisfactory viability of seed up to 5 years with ordinary dry storage . . . . contrary to some reports, there appears to be no dormancy . . . . Germinative energy [speed] at 68° to 86° F. averages 45 to 72 per cent in 25 to 41 days, and germinative capacity ranged from 55 to 100 per cent . . . . A sowing depth of one-half inch is recommended. About 33 per cent of the viable seed sown produce usable seedlings."

Exploration studies as reported by Cram (6) may be summarized as follows:

> *Caragana arborescens* was found to be a naturally cross-pollinated species, with insects of the order Hymenoptera being the principal pollinating agents. Mature open-pollinated seedlings exhibit a wide range of variation for such characteristics as plant height, habit of growth, time of foliation and defoliation, date of bloom and seed yield, etc. Artificial self-pollination could be effectively performed by "tripping" isolated flowers within a three-day period following anthesis. Self-fertility of 163 selections, when evaluated as the percentage of tripped flowers setting mature pods, ranged from 0 to 100 per cent; respective open-pollination sets varied from 2 to 95 per cent. Cheesecloth bags were found to provide adequate isolation for virgin bloom, and they proved superior to glassine or manila paper bags as manifested by the set resulting after tripping. Several seedtrees appeared to exhibit "self-tripping" tendencies, which resulted in sets of 1 to 15 per cent of non-tripped flowers. The higher percentages may be attributable to an autogamous characteristic of the plant, however, low sets might be due to pollination by the whipping action of the bags or by aphids present within the bag. Open-pollinated seed from 35 seedtrees exhibited a range of 11.1 to 35.9 mg. for average seed weight, a range of 51.2 to 88.6% for germination and a non-significant correlation of .273 between seed weight and germination capacity. Sixty per cent germination was obtained for seed harvested 15 days prior to natural dehiscence of the pods. Presence of two destructive insects—a seed chalcid and a green aphid—were reported for the species. A general similarity of some *Caragana* characteristics to those of alfalfa was noted.

Alfalfa breeding investigations have a relation to the present study. Three indices of self-fertility have been used by alfalfa breeders. They are: (1) pods set per flower tripped; (2) seeds harvested per flower tripped; and (3) seeds per pod. Wilsie and Skory (36) quote Sandal as finding "the correlation between seeds per flower tripped and pods set per flower tripped was of the order 0.9 or more." Wilsie and Skory found a range in self-fertility from 0.24 to 2.33 seeds per flower tripped, and reported a non-significant correlation between [forage] yield of open-pollinated progenies and self-fertility [of the parents] and between the yield of selfed progenies and self-fertility. Evidence was presented by Bolton and Fryer (2), which suggested the number of seeds per pod as a more reliable measure of self-fertility for "fertile" plants than the percentage of flowers tripped giving rise to pods. Later Bolton (3) reported a range from 0.02 to 5.22 seeds

per flower selfed, compared with 2.67 to 8.26 seeds per pod for cross-fertility. A slight but highly significant correlation value indicated a relation between self- and cross-fertility. Alfalfa has been found to set considerable seed without tripping of flowers (16, 17, 29, 30). Tysdal and Kiesselbach (31) state "studies have indicated that the number of seeds set per flower tripped is a better indication of the self-fertility of plants .... the more self fertile the plant the more seeds per pod when self-fertilized". A "significant positive correlation between self-fertility and seed production under poor seed-setting conditions, but a significant negative correlation under good seed-setting conditions" was found. They also report a study where plant selections were divided into three self-fertility groups; highly self-fertile (80 per cent set), medium self-fertile (48–57 per cent set) and low self-fertile or sterile (less than 31 per cent set). Analysis of the forage yields of progenies from open-pollination of these plants showed significant differences between the means of these groups. The average yield of the progeny from highly self-fertile plants was lowest and that of the low self-fertility group averaged highest. From a test where the S_1 [first inbred generation] of 54 lines was compared with the original open-pollinated varieties Tysdal *et al.* (30) reported the average forage yield of the S_1 lines to be 68 per cent of the original variety. Investigations (5, 31) have indicated that differential fertilization in favour of unrelated pollen occurred in alfalfa, when the stigma was pollinated by a mixture of self-, sib- and foreign pollen. This would normally result from insect visitations. Stewart (28) studied the effect of inbreeding on variability in alfalfa by comparing adjacent rows of the S_1 and the parent variety with 7 to 30 plants per row. He found "inbred progenies are distinctly less variable in height than the parental variety". Erickson (9) reported that higher germination and more vigorous seedlings resulted from larger seeds.

Numerous articles have been published on the relationship of seed size and vigour of the resulting plants. Kiesselbach (15) studied yields of large and small seeds of wheat and oats and reported plants from the small seed were shorter. Kotowski (18) investigated the effects of seed size of peas, beans and cabbage. Early plant growth up to blossoming stage was in direct relation with seed size, and difference attributable to seed size disappeared completely at harvest-time. Kotowski reported large cabbage seed germinated earlier and at 60 days the seedlings produced by large and small seed had the same ratio of weight as did the original seed. However, the differences between plants from different sized seeds disappeared during field growth. The relationship of seed weight to vegetative growth for plants of soybean, cucumber and tomato has been reported by Oexemann (23). Highly significant correlations were found between seed weight and dry weight as well as height of 40-day plants, but little or no relationship at maturity was reported. Leggatt and Ingalls (19) reported no significant differences in germination speed, in germination capacity, or in mean size of roots for five different sieve-size groups of turnip seed. These authors state "small seed size exhibited as high germination capacity as the large seed in laboratory tests, yet failed to give as good a stand in the field". Chang (4) reported on results of germination tests with *Juglans regia*, *Prunus persica* and *Quercus variabilis*. A direct correlation was found for the relationship of germination per cent and seedling development with size

and weight of the seeds. Righter (25) reviewed the relationship of seed and seedling size in pines and states, "there is no positive correlation between seed size and inherent vigour. The correlations between them may be regarded solely as a temporary effect due primarily to variation in endosperm size."

The effect of inbreeding and cross-breeding upon vigour in plants has been studied by scientists for many years. Those reports pertaining to the first generation are of immediate interest. Darwin (7) determined the effect of self- and cross-pollination upon the height of resulting progeny treated as paired plants for a variety of naturally cross-pollinated species. The ratio of the mean height of cross- to self-pollinated seedlings of *Sarothamnus scoparium* [broom in Leguminosae) was reported to be 100 : 46 for five pairs of seedlings of the first generation. For fifteen paired seedlings of corn a ratio of 100 : 87 and 100 : 91 for the mature plants was obtained. Darwin's data for the 15 pairs of corn plants have been analysed by Fisher (10), who applied "Students" t-test to the paired values. Fisher found the height of plants from cross-pollination to be superior to that of those from self-pollination at the 5 per cent level, though barely so. Numerous articles have been published concerning the effects of inbreeding of corn upon seed yield, but with the exception of Darwin's none deals directly with vigour of the first generation as measured by height or weight. Jones (14) reports the mean heights of four lines inbred for five successive generations as 86.7, 81.1, 90.6, and 76.5 inches compared to 117.3 inches for the parent variety.

Hayes and Immer (13) in a summary of the effects of self-fertilization in cross-pollinated crops report the following from a study in alfalfa conducted by Tysdal. Self-fertilized lines in space-planted rows of 10 to 60 plants were compared with standard varieties (i.e. open-pollinated plants) for green weight forage yields. The average yield of 54 S_1 lines was 68 per cent of the original parents, while the range was 26 to 105 per cent. Tysdal was quoted as stating "when a given plant is chosen for selfing from a mass population, there is no way of knowing whether it, itself, was the result of cross- or self-fertilization".

Beaumont studied (1) the height of apple seedlings in four progenies two and five years after they had been set out in permanent locations. Height in the second and fifth years in these locations was found to be related with correlation values ranging from .396 to .691. Beaumont concluded, height would not seem to be the only measure that must be considered if the probable vigour of a tree is to be predicted from early analyses. In this study the trees were already four years old from seed when the earlier observations were made.

MATERIAL AND METHODS

Fertility of Parental Trees

Selections were made in 1947 and 1948 from trees of *C. arborescens*, which were produced by open-pollination seed and were growing in field shelterbelts at the Forest Nursery Station, Indian Head, Saskatchewan. Trees were selected on the basis of such characteristics as height, habit of growth, time of foliation, leaf size and colour, etc. The characteristics of

the fourteen selections involved in this study are listed in Table 1. Each parent tree in this study is identified by a selection number prefixed by a number or letter designating its location as to shelterbelt. Thus, 1–1, 1–9 1–10 are selections No. 1, 9 and 10, which are growing in the No. 1 field shelterbelt. It is apparent from this that the 14 parent trees listed in Table 1 were located in six different shelterbelts on the Forestry Station. For the sake of brevity in this study, the single word caragana has been used as the common name of *C. arborescens* in place of Siberian pea-tree.

Self-fertility or fruitfulness of each selection was determined in 1948 by the following procedures. Two branches of each parent tree were enclosed in 8 × 24 in. cheesecloth bags in the early bud stage. When the tree was in "full" bloom, the bags were removed and the "fresh" flowers were "tripped" with folded 2 × 2 in. cardboard squares. All non-tripped flowers and buds were removed and the bag replaced. The number of flowers tripped, the number of mature pods and the number of seeds harvested were recorded. Three measures of self-fertility were calculated for each parent tree. These were: (1) the number of seeds harvested per flower tripped; (2) the number of mature pods harvested per flower tripped and (3) the number of seeds per pod harvested. For the sake of brevity these are referred to later as seeds/flower, pods/flower and seeds/pod, respectively. The degree of open-fertility or cross-fruitfulness of each parent tree was estimated on the basis of the "set" by open-pollination on a comparable branch. Here the number of "fresh" flowers was recorded, all others being removed. The pods set per "fresh" flower and seeds per pod harvested were calculated. In 1949 the self- and open-fertility determinations for the fourteen parent tree selections were repeated in the same manner. When possible, however, five branches were selfed and two branches were used for open-pollination checks. The relationship of the three measures of self-fertility was determined for the two years and they were evaluated as to their reliability. Similar relations were studied for open-fertility estimates, and for the relationships of self- and open-fertility values.

TABLE 1.—CHARACTERISTICS OF THE FOURTEEN CARAGANA TREES SELECTED FOR PARENT MATERIAL

Tree	Visual Characters
1–1	Early foliation and bloom, grey foliage
1–9	Mid-season foliation, pale foliage
1–10	Late defoliation, dark bloom and foliage
2–5	Late defoliation, dense slender branches
V–3	Upright growth habit, dense branching
V–10	Open-vase habit, early defoliation
V–12	Dense growth, stout branches
6–18	Tree-like habit of growth, late bloom
6–22	Vase-habit, stout branches, red shoots
16–1	Tree-like habit, stout branches, mid-season bloom
16–4	Dense stout branches, late foliation
16–11	Open-spreading habit, early foliation and bloom
16–31	Upright habit, early foliation, late bloom
21–27	Open-spreading habit, mid-season bloom, red shoots

Size of Seed

Seeds, resulting from self- and open-pollinations of fourteen trees, were classified into four diameter-size groups by passing them through U.S. standard sieves. Each group was designated with the number of the last sieve through which it passed; thus No. 8 seed had a diameter range of 2.0 to 2.3 mm.; No. 7 a range of 2.4 to 2.8 mm.; No. 6 a range of 2.9 to 3.3 mm. and No. 5 seed a diameter range of 3.4 to 4.0 mm. Samples of 40 seeds from each type of pollination were taken from both the No. 6 and No. 7 size group of each parental tree for a germination study and the individual weight of each seed was recorded to the nearest milligram. The relationship of seed weight to "set" was investigated in 1949.

Germination of Self- and Open-pollination Seed

The above mentioned 40-seed samples were used to study amount and speed of germination for the 14 parent trees, for seed of two sizes, and for seed from self- and open-pollination. The samples of 40 seed were each divided into four sub-samples. The germination study was in the form of a split-plot design with four replications, and it was conducted in the greenhouse in 1949. Each replication or block was divided into two long main plots, one being sown to seed of one size and one sown to seed of the other size. Main plots were divided into 14 sub-plots to which seed of the 14 trees was assigned at random. Each sub-plot consisted of two adjacent rows, one being sown to 10 seeds from self-pollination and the other to 10 seeds from open-pollination. Each seed was placed in an individual $2 \times 2 \times 2$ in. plant band at a depth of half an inch, on April 28. The planting arrangement for sizes of seed, trees [sources of seed] and seed resulting from the two types of pollination, was at random. Records were maintained of the identity of each seed, its weight, and the number of days to emergence. From these data the amount and speed of germination were determined for the first 25 days following sowing. The relationship between self-fertility of parental trees and the amount and speed of germination of their seed was calculated.

Vigour of Seedling Progenies

Seedlings obtained from the germination study were transplanted outdoors to compare the vigour of one-year-old plants in relation to characteristics of parent trees. Seedlings, resulting from seed produced by self- and open-pollination of the 14 parent trees, were planted June 18 in adjacent rows spaced 4×4 inches. Since disease had reduced the number of seedlings drastically there was no replication. Two measures of seedling vigour were studied. The height of each seedling was recorded in centimeters, and the weight of each dormant seedling was determined in grams at the end of the 1949 growing season. Significance of differences in height and weight of progenies was determined, first by an unweighted analysis of variance; then means were compared by utilizing standard errors of means. The relationship of the two measures of seedling vigour was determined, and in addition their relationships to seed weight, germination speed of the seed, and self-fertility of the parental trees were calculated.

In reporting the statistical results of this study a single asterisk (*) is used to indicate significance at the 5 per cent level, and a double asterisk (**) to indicate significance at the 1 per cent level, unless otherwise stated.

For the purpose of brevity in tables, the set, seeds and seedlings resulting from self-pollination are designated by "⊗", similarly from open-pollination by "O". For the same reaason, types of pollination have been implied by "pollens", and parental source of seed by "tree".

EXPERIMENTAL RESULTS

Fertility of Parental Trees

The fertility of each tree as determined in 1948 and 1949 is given in Table 2, where parental trees are listed in descending order of their mean seeds/flower values. Pods/flower and seeds/flower values of the 14 parent trees appeared to be relatively consistent in the two years, as indicated by correlation values of .645 and .782, respectively [1 per cent level of r for 12 D.F., = .661]. However, seeds/pod values exhibited marked differences for some trees in the two years and gave a correlation value of only .464. At the same time, pods/flower and seeds/flower values for the 14 trees were associated with correlation values of .701 in 1948 and .783 in 1949. The relationships of seeds/pod with pods/flower, and of seeds/pod with seeds/flower, were not significant at the one per cent level in either year, as indicated by their respective correlation values of .600 and .527 in 1948 and .353 and .608 in 1949. Although pods/flower is a convenient measure of self-fertility for *C. arborescens*, it appears that seeds/flower provides a more reliable and consistent index of this character. In this study the parental trees have been assigned the self-fertility rating of their mean seeds/flower as listed in Table 2. On this basis self-fertility of the parent trees ranged from 0.37 to 2.51 seeds per flower tripped, or in other words from low to high.

Cross- or open-fertility could only be estimated from the pod-set and seeds per pod values resulting under open-pollination, as the actual number of flowers tripped [i.e. visited] by pollinating insects was unknown. Results obtained in 1948 and 1949 are given in Table 3, with parental trees listed in

TABLE 2.—SELF-FERTILITY OF 14 CARAGANA TREES AS DETERMINED BY THREE MEASURES IN 1948 AND 1949

Parent Tree	Seeds/Pod		Pods/Flower		Seeds/Flower		
	'48	'49	'48	'49	'49	'49	Mean
16–31	2.0	1.3	0.29	0.11	0.59	0.14	0.37
1–10	2.3	1.0	0.21	0.33	0.47	0.33	0.40
V–3	2.1	2.0	0.20	0.22	0.44	0.44	0.44
V–12	1.6	2.2	0.54	0.28	0.83	0.61	0.72
V–10	1.5	3.0	0.58	0.23	0.87	0.65	0.76
6–22	2.6	2.5	0.36	0.34	0.92	0.86	0.89
1–9	2.7	2.5	0.49	0.32	1.34	0.73	1.03
21–27	3.2	3.6	0.58	0.11	1.87	0.63	1.25
2–5	3.0	3.5	0.47	0.39	1.40	1.40	1.40
16–4	1.6	1.7	1.00	0.84	1.61	1.44	1.52
6–18	2.8	3.5	0.63	0.52	1.75	1.71	1.73
1–1	3.2	3.4	0.67	0.51	1.92	1.72	1.82
16–11	2.5	2.5	0.82	1.00	2.05	2.47	2.26
16–1	2.6	3.4	0.98	0.72	2.53	2.50	2.51
Means	2.4	2.6	0.56	0.42	1.33	1.12	

TABLE 3.—POD AND SEED SET UNDER OPEN-POLLINATION IN 1948 AND 1949 FOR 14 CARAGANA TREES

Parent Tree	Seeds/Pods			Pods/Flower		
	1948	1949	Mean	1948	1949	Mean
V–12	2.8	3.9	3.3	.66	.31	.48
V–10	3.9	4.2	4.0	.49	.41	.45
2–5	5.6	4.4	5.0	.53	.36	.44
16–31	3.3	3.4	3.3	.73	.09	.41
16–1	1.9	2.6	2.2	.59	.20	.39
6–18	2.8	4.8	3.8	.70	.08	.39
1–10	1.9	3.0	2.4	.55	.20	.37
V–3	3.6	3.4	3.5	.39	.27	.33
1–1	5.4	2.4	3.9	.49	.17	.33
1–9	3.3	4.3	3.8	.57	.08	.32
6–22	3.2	4.2	3.7	.30	.32	.31
21–27	4.5	2.8	3.6	.46	.08	.27
16–11	2.4	2.7	2.5	.16	.32	.24
16–4	2.8	1.9	2.3	.11	.21	.16
Means	3.4	3.4		.48	.22	

descending order of their mean pods/flower values. Inter-annual correlations were not significant for either of the two estimates of open-fertility, which would suggest that they are neither consistent nor reliable estimates of the character. In addition a negative non-significant correlation value of $-.300$ was found between self-fertility and open-fertility [pods/flower] for 1948; and a positive non-significant correlation of .126 in 1949. This would suggest that no association was exhibited between self-fertility and open-fertility for the two years. However, the mean fruitfulness based on pods/flower values in Tables 2 and 3 was apparently less in 1949 than in 1948 for both self-pollination and open-pollination. Aphids, which were present in large numbers within the selfing-bags in 1948, may have acted as pollinators to a limited extent resulting in a higher set in 1948. Nevertheless, the greater set in 1948 by open-pollination could not be attributed to aphids to the same degree, due to the fact that aphids were less prevalent outside the selfing-bags. As a result it would appear that the lower set for 1949 may be entirely or partly due to floral injury resulting from a temperature of 22° F. which occurred May 23 several days after selfing had been completed. The comparative pod "sets" for the two years would seem to suggest that the cheesecloth selfing-bags may have provided some protection from the frost.

Size of Seed

When all seeds resulting from self-pollination and open-pollination of 14 parent trees were sieved, it was found that 90 per cent of the self-pollination seed and 88 per cent of the open-pollination seed fell into the combined No. 6 and No. 7 sizes. It was for this reason that seeds of these two sizes were selected for the germination test. The weight data for the seed utilized in the germination study are summarized in Table 4, where trees are listed in descending order of their mean seed-weight values. Due to heterogeneity of these data, a combined analysis seemed erroneous and

TABLE 4.—WEIGHT IN MILLIGRAMS OF THE 1948 SEED OF TWO SIZES FROM SELF- AND OPEN-POLLINATION OF 14 CARAGANA TREES
(Samples of 40 seed of each pollen and size)

Parent Tree	Pollination	Size No. 6 (2.9–3.3 mm.)	Size No. 7 (2.4–2.8 mm.)	Means	Means for Tree
16–4	⊗	29.7	23.5	26.6 ± 0.5	
	○	28.4	23.5	24.5 ± 0.6	25.5 ± 0.4
V–12	⊗	28.8	21.5	25.1 ± 0.5	
	○	27.8	22.1	24.9 ± 0.5	25.0 ± 0.4
16–1	⊗	31.8	20.3	26.1 ± 0.8	
	○	27.2	19.7	23.5 ± 0.6	24.8 ± 0.5
16–31	⊗	29.1	19.8	24.4 ± 0.7	
	○	27.5	20.8	24.2 ± 0.5	24.3 ± 0.4
1–10	⊗	24.5	19.5	22.0 ± 0.4	
	○	32.0	19.9	26.0 ± 0.8	24.0 ± 0.5
V–3	⊗	28.2	18.5	23.3 ± 0.7	
	○	27.0	19.2	23.4 ± 0.6	23.3 ± 0.4
V–10	⊗	26.4	20.3	23.4 ± 0.5	
	○	27.0	18.5	22.8 ± 0.6	23.1 ± 0.4
6–22	⊗	27.1	19.8	23.4 ± 0.6	
	○	26.1	19.0	22.5 ± 0.6	22.9 ± 0.4
2–5	⊗	26.5	19.4	22.9 ± 0.6	
	○	26.8	18.3	22.5 ± 0.7	22.7 ± 0.4
16–11	⊗	24.8	20.3	22.5 ± 0.5	
	○	24.4	17.6	21.0 ± 0.5	21.7 ± 0.4
21–27	⊗	24.2	17.2	20.7 ± 0.5	
	○	25.3	19.3	22.3 ± 0.5	21.5 ± 0.4
1–1	⊗	23.6	18.9	21.2 ± 0.4	
	○	22.7	18.9	20.8 ± 0.3	21.0 ± 0.3
6–18	⊗	22.7	15.6	19.1 ± 0.5	
	○	22.5	17.1	19.8 ± 0.4	19.4 ± 0.3
1–9	⊗	20.5	16.9	18.7 ± 0.3	
	○	22.2	16.1	19.1 ± 0.4	18.9 ± 0.3
Means	⊗	26.3	19.4	22.8 ± 0.011	
	○	26.3	19.4	22.7 ± 0.011	

standard errors were determined for each 80-seed sample. Comparisons were, therefore, made on the basis of different standard errors of the means. Differences significant at the 5 per cent level are apparent between weights of seed from various trees and between self-pollination seed and open-pollination seed of some trees. Seed from two trees, 6–18 and 1–9, proved lighter than that from all other trees with the exception of 1–1. One tree, 16–4, produced the heaviest seed with a mean weight of 25.5 milligrams. Differences in weight are exhibited between self-pollination and open-pollination seed from five trees. These differences were in favour of open-pollination seed for parental trees 1–10 and 21–27; while self-pollination

seed from 16–1, 16–4 and 16–11 were heavier than the open-pollination seed, However, it should be noted that the mean weight of the self-pollination seed equalled that of the open-pollination.

Data on seed set and seed size obtained in the 1949 seed-year were utilized to estimate the relationship between these two characters. The degree of set of seeds/pod, resulting from self- and open-pollination, was found to bear no significant relation to the average weight of the seed so produced. Thus it would appear that seed size is not directly attributable to the amount of seed produced.

Germination of Self- and Open-pollination Seed

The amount and speed of germination, for seed of two sizes resulting from self- and open-pollination of 14 trees in 1948, was investigated in the greenhouse in 1949. However, because three of the four subplots of seed from parent tree 16–1 failed to germinate, the following analyses were confined to results obtained for seed from the remaining 13 trees only.

Analysis of variance for the amount of germination is given in Table 5, where types of pollinations have been designated as pollinations for the sake of brevity. Sub-plot values were the total number of seeds that germinated of the ten planted. Seeds of the different sizes show no significant differences in their germination ability. However, significant differences between seed from the various parental trees and between self- and open-pollination seed are suggested. Significance was also indicated for the P × S interaction, which would suggest that seeds of the two sizes were differential in their germination capacity for the two types of pollination. Amount of germination varied from 4.1 to 7.4 for the 13 parental trees, or in other words germination capacity ranged from 41 to 74 per cent. The germination capacity obtained for seed from each tree was as follows: 6–18 = 74; 2–5 = 71; 1–10 = 69; 1–9 = 65; 16–1 = 62; 21–27 = 54; V–3 = 53; 1–1 = 50; V–12 = 49; 16–31 = 42 and for V–10, 6–22 and 16–4 = 41 per cent. The difference necessary for significance at the 1 per cent level was 18 per cent. Hence, seed from trees 6–18, 2–5 and

TABLE 5.—ANALYSIS OF VARIANCE OF AMOUNT OF GERMINATION IN 25 DAYS FOR SELF- AND OPEN-POLLINATION SEED OF TWO SIZES FROM 13 CARAGANA TREES

Source of Variation	D.F.	Mean Square	F.
Replications	3	14.776	0.50
Sizes	1	152.327	5.19
Error (*a*)	3	29.365	
Trees	12	23.277	6.34**
T × S	12	5.952	1.62
Error (*b*)	72	3.671	
Pollinations	1	9.308	5.14*
P × S	1	9.308	5.14*
P × T	12	3.016	1.74
P × T × S	12	1.662	0.92
Error (*c*)	78	1.811	

TABLE 6.—AMOUNT OF GERMINATION FOR SEED OF TWO SIZES AND OF TWO TYPES OF POLLINATION

(Means of 52 sub-plots of 10 seed each)

Type of pollination	Size No. 6 (2.9–3.3 mm.)	Size No. 7 (2.4–2.8 mm.)	Mean**
Self*	6.81	4.67	5.74
Open	5.96	4.67	5.34
Means of Sizes	6.38	4.67	

* Significant difference between pollens for same size of seed, 5 per cent level, 0.53.
** Significant difference between means of pollens at 5 per cent level, 0.37.

1–10 exhibited superior germination to that from all but four other trees; while seed of 16–4, 6–22, V–10 and 16–31 demonstrated inferior germination to that of seed from all but four trees. It is evident that parental trees differ in the germination capacity of their seeds.

Germination data for types of pollination and sizes of seed have been summarized in Table 6. It is apparent that the germination capacity of the smaller seed was the same for both types of pollination; while for the larger size, seed from self-pollination exhibited a superior germination of 8 per cent over open-pollination seed. Thus it is evident that the significant P × S interaction was all due to the differences provided by large seed. It is also apparent in Table 6 that germination capacity of seed produced by self-pollination was superior to that of open-pollination seed, with 57 and 53 per cent respectively. The difference in germination capacity between large seed with 64 per cent and small seed with 47 per cent was not significant. However, due to the experimental design, precision on the effect of seed size was lower than that for other main effects. Nevertheless, results obtained in this study indicate that both source of seed and type of pollination influenced germination capacity of caragana seed.

Germination time of a seed was taken as the number of days from seeding to emergence of the seedling. As this character of seed is often designated as germination speed in literature, the two terms are considered and used synonymously in this study. This character was investigated as a possible measure of early vigour of the progeny. Data on germination speed were obtained by averaging the respective days-to-emergence values of each sub-plot for the first 25 days following seeding. Analysis of variance of the data on germination speed appears in Table 7. The analysis would suggest that size of seed had no measurable effect upon germination speed of seed in this study. Significant differences are indicated between seedtree sources of seed, and significance is also indicated for the T × S interaction.

The mean germination speed for seed of the two sizes from 13 parental trees is shown in Table 6. It is apparent that germination speed of seed from the 13 parental trees differed significantly with a range of 11.6 to 14.8 days. Seed from V-12 exhibited slower germination than that of seed from nine trees, while seed from 16–11 and 6–18 demonstrated faster germination than that of eight trees. Seed of the two sizes provided differential germination speed in the case of only two parental trees: large seed of 1–9 and V–3

TABLE 7.—ANALYSIS OF VARIANCE OF GERMINATION SPEED FOR SELF- AND OPEN-POLLINATION SEED OF TWO SIZES FROM 13 CARAGANA TREES

Source of Variation	D.F.	Mean Square	F.
Replications	3	26.211	2.09
Sizes	1	47.215	3.76
Error (*a*)	3	12.541	
Trees	12	13.727	4.15**
T × S	12	7.473	2.26*
Error (*b*)	72	3.304	
Pollinations	1	6.196	2.08
P × S	1	7.200	2.42
P × T	12	4.945	1.66
P × T × S	12	3.548	1.19
Error (*c*)	78	2.973	

TABLE 8.—GERMINATION SPEED FOR SEED OF TWO SIZES FROM 13 PARENTAL TREES OF CARAGANA

(Mean days-to-emergence values for 8 subplots)

Parental Tree	Size No. 6 (2.9–3.3 mm.)	Size No. 7 (2.4–2.8 mm.)	Mean for Tree ***
	(days)	(days)	(days)
V–12*	14.1**	15.5	14.8
16–4	14.4	13.7	14.0
1–1	13.9	14.2	14.0
21–27	13.8	14.2	14.0
1–9	12.1	15.4	13.7
6–22	13.5	13.9	13.7
16–31	13.7	13.3	13.5
V–10	13.9	12.3	13.1
1–10	12.2	13.6	12.9
2–5	11.7	13.9	12.8
V–3	11.5	13.9	12.7
16–11	10.9	12.5	11.7
6–18	10.8	12.4	11.6
Means for Sizes	12.8	13.8	

* Significant difference between sizes for same tree, 5 per cent level, 2.3 days.
** Significant difference between trees for same size, 5 per cent level, 1.8 days.
*** Significant difference between means for trees at the 5 per cent level, 1.3 days.

germinated earlier than the respective small seed. For the large size it was apparent that seed of 16–11 and 6–18 germinated earlier than that of seven other trees; but for the small seed size that of 16–11, 6–18, and V–10 provided earlier germination to that of only two trees. It was apparent that size of seed has no general effect upon germination speed of the seed. Similarly, it would appear that type of pollination had little if any effect upon germination speed of seed. Further, no association was apparent between self-fertility of parental trees and germination speed of either their self-pollination seed or their open-pollination seed. Although this study is based on relatively small samples, it seems that germination speed for seed of *C. arborescens* is not materially influenced by any of the parental tree characteristics involved in this study.

Vigour of Seedling Progenies

Seedlings resulting from the preceding germination study were transplanted outdoors for study of their vigour. Numerous seedlings were attacked by a root-rot organism [*Fusarium solani**] before completion of the germination. Nevertheless, in order to obtain an estimate of the relationship of seed size and type of pollination to seedling vigour all possible seedlings were transplanted on June 18. Self- and open-pollination seedlings resulting from each seed size and each tree were planted in adjacent plots. The numbers of self-pollination seedlings and of open-pollination seedlings in each pair of plots were identical. However, the total number of plants for each of the different trees and for each of the two seed sizes varied from 17 to 41 and from 4 to 26, respectively. Favorable growing conditions were terminated by a killing frost on September 25. Thus, the growing season for these seedlings, from sowing to frost, was approximately 150 days. Two measures of plant size were selected to evaluate seedling vigour, namely height and weight. Height of each seedling was determined September 25, as the distance in centimetres from the lowest node to the apex. All seedlings were lifted and placed in storage on October 7. Weight of each seedling was recorded in grams on February 1, when dormancy was suggested by complete defoliation.

Unequal numbers in the classes for seed sizes and trees, and the absence of replications, imposed restrictions upon the method of analysis for the vigour data. An analysis of variance for the combined data, computed from the means of classes, was assumed to provide approximate error estimates applicable to main effects. The variance being used for error is that of the residual, which is essentially that of the triple interaction. Such an analysis for the combined height data is given in Table 9. Means of the 56 classes form the basis of this analysis, with each mean being considered a degree of freedom. Tests for significance of the variances in Table 9 were conducted in the following manner. Residual variance was used to test that of each interaction and each main effect, with the results listed in Table 9. Thus significance was indicated for trees, sizes, pollinations,

* Identification courtesy of W. E.Sackston.

TABLE 9.—UNWEIGHTED ANALYSIS OF VARIANCE FOR HEIGHT OF SELF- AND OPEN-POLLINATION SEEDLINGS FROM SEED OF TWO SIZES OF 14 CARAGANA TREES

(Means of classes in centimetres)

Source of Variation	D.F.	Mean Square	F.
Trees	13	29.82	10.57**
Seed Sizes	1	197.51	7.04**
T × S	13	7.49	2.66*
Pollinations	1	195.19	69.22**
P × T	13	9.11	3.23*
P × S	1	0.40	0.14
Residual	13	2.82	
Total	55		

T × S, and P × T. However, the two interactions were significant only at the 5 per cent level, while all main effects were indicated as being significant at the 1 per cent level. Hence, the existence of significant differences were suggested between heights of seedlings for some parental trees, for the two seed sizes, and for the two types of pollination. In addition there are indications that some parental trees may have produced progenies which were differential in height for the two seed sizes as well as for the two types of pollination. These results indicate the need for further examination of the data by more critical and adequate methods.

The mean heights of seedlings for the two seed sizes, for the two types of pollination, and for the 14 parental trees are shown in Table 10. Standard

TABLE 10.—HEIGHT OF SEEDLINGS RESULTING FROM SEED OF TWO SIZES AND FROM SELF- AND OPEN-POLLINATION OF 14 CARAGANA TREES

Parent Tree	Seed Size	Height of Seedlings		Pollination	Height of Seedlings		Mean for tree
		(No.)	(cm.)		(No.)	(cm.)	(cm.)
21–27	6	30	25.3 ± 1.7	⊗	29	21.5 ± 1.5	
	7	28	20.2 ± 1.7	○	29	24.5 ± 1.6	22.8 ± 1.1
V–12	6	50	21.4 ± 0.9	⊗	41	19.4 ± 0.9	
	7	32	20.5 ± 1.1	○	41	22.5 ± 1.0	20.9 ± 0.7
16–4	6	22	21.3 ± 1.5	⊗	22	19.6 ± 1.3	
	7	22	18.4 ± 1.3	○	22	20.1 ± 1.3	19.8 ± 0.9
V–10	6	34	21.3 ± 1.4	⊗	25	15.6 ± 1.1	
	7	16	17.7 ± 2.1	○	25	23.4 ± 1.7	19.5 ± 1.1
2–5	6	52	20.9 ± 1.1	⊗	40	16.4 ± 1.2	
	7	28	16.2 ± 1.3	○	40	20.7 ± 1.2	18.5 ± 0.9
6–22	6	34	17.6 ± 1.1	⊗	26	16.2 ± 1.0	
	7	18	16.8 ± 1.3	○	26	18.2 ± 1.2	17.2 ± 0.8
16–1	6	38	17.4 ± 1.2	⊗	25	16.0 ± 1.4	
	7	12	16.3 ± 1.6	○	25	17.7 ± 1.3	16.8 ± 0.9
16–11	6	46	18.3 ± 0.9	⊗	34	15.7 ± 1.0	
	7	22	14.0 ± 1.4	○	34	16.6 ± 1.3	16.2 ± 0.8
16–31	6	36	19.5 ± 1.6	⊗	23	13.1 ± 1.0	
	7	10	12.8 ± 2.5	○	23	19.2 ± 2.4	16.1 ± 1.4
6–18	6	52	17.8 ± 1.0	⊗	39	14.4 ± 0.9	
	7	26	13.9 ± 1.1	○	39	17.3 ± 1.2	15.8 ± 0.8
V–3	6	44	20.5 ± 1.8	⊗	30	10.1 ± 1.7	
	7	16	11.1 ± 2.0	○	30	21.5 ± 2.0	15.8 ± 1.5
1–10	6	46	16.5 ± 1.0	⊗	40	14.1 ± 1.1	
	7	34	14.0 ± 1.1	○	40	16.4 ± 1.1	15.3 ± 0.8
1–9	6	42	14.5 ± 1.0	⊗	29	12.1 ± 0.9	
	7	16	14.8 ± 2.1	○	29	17.2 ± 1.5	14.7 ± 0.9
1–1	6	26	16.0 ± 1.2	⊗	17	11.8 ± 1.7	
	7	8	9.2 ± 1.2	○	17	13.3 ± 1.4	12.6 ± 1.1
Mean	6	552	19.2 ± 0.3	⊗	420	16.1 ± 0.3	
	7	288	15.4 ± 0.4	○	420	20.0 ± 0.4	

errors of means were not obtained from the variances in Table 9, but were calculated independently from the seedlings in each plot by combining the appropriate data for seed sizes or for types of pollinations. In the table, parental trees are listed in descending order based on the mean heights of their progenies.

It is evident from the data in Table 10 that seedlings from large seed were generally more vigorous than those from small seed, and the difference was also significant for some parental trees. Seedlings resulting from the large seed [No. 6 sieve size, 2.9 to 3.3 mm. in diameter] demonstrated 25 per cent more height than seedlings from small seed [No. 7 size, 2.4 to 2.8 mm.] on the average. A similar trend was evident for the progenies of all except one tree [1–9]. The height difference in favor of seedlings from large seed was significant for seven parental trees: 21–27, 16–11, 16–31, V–3, 1–10, and 1–1. Thus it appears that diameter or sieve size of seed has a general and direct influence upon height of 150-day caragana seedlings. This suggests the possibility of a relationship between size of embryo and vigour of the resulting seedlings.

It is also evident from the data appearing in Table 10 that progenies from open-pollination seed proved more vigorous than those from self-pollination seed. Mean height of bulked self-pollination seedlings was only 80 per cent of that for open-pollination seedlings, which gives a growth ratio of 80 : 100 for the 150-day plants of caragana. Open-pollination progenies of seven parental trees—V–12, V–10, 2–5, 16–31, 6–18, V–3 and 1–9—exhibited superior height to that of the progenies from self-pollination. A similar trend was apparent for progenies of the remaining trees, although not to a significant degree. Therefore, the first inbred generation of caragana would seem to manifest a loss in vigour typical of cross-pollinated species of plants.

Comparison of the inbred progenies of the 14 parent trees revealed that the self-pollination seedlings of 21–27 were superior in height to those of all trees except 16–4 and V–12. Self-pollination seedlings of these two trees exhibited greater height than those of nine trees. On the other hand, seedlings resulting from open-pollination of tree 21–27 demonstrated superior height to those from nine trees, but not to those of V–12, V–10, 2–5 and V–3. Open-pollination progenies of V–10 and V–12 ranked second and third for vigour by showing superiority to six other progenies. Thus, based on performance of open progenies, 21–27, V–10, and V–12 appear as superior parental trees; while on the basis of inbred progenies, 21–27, 16–4, and V–12 seemed to provide superior parental material.

TABLE 11.—UNWEIGHTED ANALYSIS OF VARIANCE FOR WEIGHT OF SELF- AND OPEN-POLLINATION SEEDLINGS FROM SEED OF TWO SIZES OF 14 CARAGANA TREES
(Means of classes in grams)

Source of Variation	D.F.	Mean Square	F.
Trees	13	2.408	4.94**
Seed Sizes	1	10.073	20.67**
T × S	13	0.762	1.56
Pollinations	1	20.100	41.26**
P × T	13	1.226	2.52
P × S	1	0.034	0.07
Residual	13	0.4872	

Analysis of weight data was conducted in the same manner as that for height. The analysis of variance of the combined data is given in Table 11. When tested with the residual variance all three interactions were found to yield non-significant F-values. By using residual variance as error, differences significant at the 1 per cent level are suggested as existing between trees, seed sizes, and types of pollinations.

Table 12 was prepared to show the mean weights of seedlings for the two seed sizes and for the two types of pollination, as well as for each of the 14 parental trees. A marked similarity in progeny vigour, as manifested by seedling height and by seedling weight, is readily apparent when the results in Tables 10 and 12 are compared. Sieve size of seed had a direct

TABLE 12.—WEIGHT OF SEEDLINGS RESULTING FROM SEED OF TWO SIZES AND FROM SELF- AND OPEN-POLLINATION OF 14 CARAGANA TREES

Parent Tree	Seed Size	Weight of Seedlings		Pollination	Weight of Seedlings		Mean for tree
		(No.)	(gm.)		(No.)	(gm.)	(gm.)
21–27	6	30	6.0 ± 0.6	⊗	29	4.4 ± 0.5	
	7	28	4.6 ± 0.4	○	29	6.2 ± 0.6	5.3 ± 0.4
V–12	6	50	4.4 ± 0.4	⊗	41	3.6 ± 0.3	
	7	32	4.6 ± 0.4	○	41	5.4 ± 0.4	4.5 ± 0.3
16–4	6	22	4.3 ± 0.5	⊗	22	4.4 ± 0.5	
	7	22	4.4 ± 0.5	○	22	4.3 ± 0.5	4.3 ± 0.3
V–3	6	44	5.4 ± 0.8	⊗	30	2.2 ± 0.6	
	7	16	2.6 ± 0.5	○	30	5.8 ± 0.9	4.0 ± 0.6
6–22	6	34	3.6 ± 0.3	⊗	26	3.6 ± 0.3	
	7	18	3.6 ± 0.4	○	26	3.7 ± 0.4	3.6 ± 0.2
V–10	6	34	4.1 ± 0.4	⊗	25	2.4 ± 0.3	
	7	16	2.8 ± 0.7	○	25	4.5 ± 0.5	3.4 ± 0.3
2–5	6	52	4.0 ± 0.4	⊗	40	2.6 ± 0.3	
	7	28	2.6 ± 0.3	○	40	4.1 ± 0.4	3.3 ± 0.3
6–18	6	52	3.9 ± 0.3	⊗	39	2.8 ± 0.3	
	7	26	2.8 ± 0.4	○	39	3.9 ± 0.4	3.3 ± 0.3
16–31	6	36	3.9 ± 0.4	⊗	23	2.1 ± 0.2	
	7	10	2.7 ± 0.6	○	23	4.5 ± 0.5	3.3 ± 0.4
16–1	6	38	3.5 ± 0.4	⊗	25	3.1 ± 0.5	
	7	12	3.0 ± 0.6	○	25	3.4 ± 0.4	3.2 ± 0.3
1–10	6	46	3.5 ± 0.4	⊗	40	2.9 ± 0.4	
	7	34	3.0 ± 0.3	○	40	3.6 ± 0.4	3.2 ± 0.3
16–11	6	46	3.6 ± 0.3	⊗	34	3.1 ± 0.2	
	7	22	2.7 ± 0.3	○	34	3.2 ± 0.4	3.1 ± 0.2
1–9	6	42	2.4 ± 0.3	⊗	29	2.0 ± 0.2	
	7	16	3.0 ± 0.6	○	29	3.5 ± 0.4	2.7 ± 0.2
1–1	6	26	2.8 ± 0.4	⊗	17	2.2 ± 0.5	
	7	8	1.6 ± 0.3	○	17	2.2 ± 0.3	2.2 ± 0.3
Mean	6	552	4.0 ± 0.04	⊗	420	3.1 ± 0.11	
	7	288	3.1 ± 0.04	○	420	4.4 ± 0.14	

influence upon the weight as well as the height of the resulting 150-day progenies. Mean weight of the combined progenies from the small seed [No. 7, 2.4 to 2.8 mm.] was only 77 per cent of that for progenies from the large seed [No. 6, 2.9 to 3.3 mm.]. Average weight of the self-pollination progenies in Table 12 was only 70 per cent of that for open-pollination progenies, giving a weight growth ratio of 70 : 100 for the 150-day caragana seedlings.

In Table 12 open-pollination seedlings demonstrated greater weight than self-pollination seedlings for all except six parental trees. Open-pollination seedlings of 21–27 were superior to those from eleven trees, although not to those of V–12 and V–3. Seedlings of 21–27 and 16–4 resulting from self-pollination exhibited greater weight than those from nine other trees. On the basis of mean weight of their self-pollination progenies, parental trees 21–27 and 16–4 offer promise as breeding material; while on the basis of open-pollination progenies 21–27, V–12, and V–3 appear desirable.

It should be made clear that the author does not consider the vigour data from this study entirely conclusive, due to the fact that some populations were too small for a satisfactory progeny test. It is possible that a residual effect of the disease which was present during germination still exists. This residual effect, especially if caused by the loss of less vigorous plants from some progenies, would result in the presentation of an incomplete picture for some parental trees. Also, the absence of replications of different treatments in a randomized manner does not eliminate the possibility that soil heterogeneity affected growth and variability of the seedlings.

Relationship of Parent Tree and Seedling Characteristics

Table 13 lists correlation values obtained for the inter-relationships of seed and seedling characteristics for 14 parental trees. The relationships for each parental tree were determined from the data of individual seeds and their corresponding seedlings; while mean values for each parental tree were used to calculate the average relationships given at the bottom of Table 13.

Height and weight of progeny were found to be associated to a high degree for each parent, as indicated by a positive and highly significant correlation value. This relationship suggests that each measure provides a similar estimate of vigour for 150-day seedlings.

The association of seed weight with germination speed attained a significant level for self-pollination seed of two trees, V–3 and 1–9; and for open-pollination seed of two trees, 21–27 and 1–10. However, as there appears to be no relation between germination speed and seedling height, the associations in these cases appear of no particular value. Nevertheless germination speed of seed was significantly associated with height of the resulting seedlings for certain parental trees. This relationship was significant for the open-pollination seed and seedlings of V–3, and for both self- and open-pollination seedlings of 6–18 and 16–4. Hence it would appear that earlier germination may be responsible for the vigour expressed by self-pollination seedlings of 16–4. However, open-pollination seedlings of V–3 exhibited only mediocre vigour [Table 10], and those of 16–4 and 6–18

TABLE 13.—SIMPLE CORRELATION COEFFICIENTS FOR THE INTER-RELATIONSHIPS OF THE SELF- AND OPEN-POLLINATION SEED AND SEEDLING CHARACTERISTICS OF 14 CARAGANA TREES

Parent Tree	Pollination	Characteristics			
		Seed Weight and Germ. Speed	Seed weight and Sdlg. Height	Germ. Speed and Sdlg. Height	Sdlg. Height and Sdlg. Weight
16–31	⊗	.309	.380	−.065	.816***
	○	.024	.715***	.255	.892***
1–10	⊗	.021	.217	−.092	.910***
	○	−.388*	.112	−.167	.905***
V–3	⊗	−.420*	.435*	−.222	.925***
	○	.125	.491**	−.388*	.905***
V–12	⊗	−.054	.362*	−.271	.809***
	○	−.250	.210	−.152	.827***
V–10	⊗	−.222	.314	−.180	.963***
	○	.024	.260	−.011	.880***
6–22	⊗	−.301	−.018	−.180	.895***
	○	.021	.179	−.055	.895***
1–9	⊗	−.480***	.129	−.120	.893***
	○	−.300	.217	.086	.996***
21–27	⊗	−.151	.412*	−.221	.953***
	○	−.402*	.687**	−.314	.899***
2–5	⊗	−.203	.186	−.258	.915***
	○	−.123	.274	.087	.929***
16–4	⊗	−.084	.570**	−.494*	.706***
	○	−.231	.209	−.574***	.646**
6–18	⊗	−.106	.248	−.335*	.842***
	○	−.137	.181	−.327*	.913***
11–1	⊗	.196	.301	−.412	.940***
	○	−.406	.426	−.176	.914***
16–11	⊗	−.144	.377*	−.281	.924***
	○	.324	.104	−.211	.833***
16–1	⊗	−.137	.010	−.043	.946***
	○	−.050	−.088	−.193	.890***
Means	⊗	.084	.210	.506	.873***
	○	−.256	.308	.092	.960***

* Significant r, 5 per cent level.
** Significant r, 1 per cent level.
*** Significant r, 0.1 per cent level.

even less. Thus, it would appear that germination speed [days to emergence] is not a factor contributing to the vigour of 150-day seedlings of caragana.

Weight of open-pollination seed and height of the resulting seedlings were found to be associated by significant correlation values for parental trees 16–31, V–3, and 21–27. A similar association was apparent for the self-pollination seed and seedlings of V–3, V–12, 21–27, 16–4, and 16–11.

These relationships suggest that the heavier seed manifested itself by producing more vigorous seedlings than those produced from the lighter seed. In the present study, parental trees 21–27, 16–4, and V–12 were found to produce the most vigorous self-pollination progenies [Table 10] while 21–27 produced the most vigorous open-pollination progeny. Therefore, it would appear that seed weight was a factor influencing the vigour of the 150-day seedlings from those selections of caragana.

The relationships of seed and seedling characteristics fail to explain any reason for the superior vigour demonstrated by open-pollination seedlings of parent trees V–10 and V–12. It would appear that superior height of these two progenies may be due to some other phenomenon. One possibility is that the low self-fertility of these two parental trees has resulted in a majority of the open-pollination seed being from cross-pollination. Cross-pollination plus the inherent vigour of trees V–10 and V–12 may explain the vigour shown by their open-pollination progenies. If this is so, the vigour of open-pollination progenies may also be a rough estimation of combining ability of the parental trees. This would suggest an association between self-fertility of parent trees and vigour of their open-pollination progenies. However, a non-significant correlation value of $-.360$ [5 per cent level of $r = .532$] was obtained for this relationship for the 14 parental trees in this study. It appears that self-fertility of the parental trees was not associated with height of their 150-day open-pollination seedlings.

DISCUSSION

As stated in the introduction, the main objective of this study was to determine the effect of parent tree characteristics upon the vigour of one-year-old seedlings of *Caragana arborescens*.

The data obtained in this study suggest that no close relation exists between: (1) the degree of pod or seed set and the size of the seed resulting from either self- or open-pollination; (2) self-fertility of the parent tree and the amount or speed of germination of either self- or open-pollination seed; (3) self-fertility of the parent trees and the vigour of their 150-day progenies from either self-pollination seed or open-pollination seed.

Although the 14 parental trees represent a very small sample of the population for a cross-pollinated species, striking differences in self-fertility of trees, germination capacity of their seed, vigour of their progenies, and effects of seed size and type of pollination upon vigour of progeny, were obtained. Nevertheless, the value of such differences in an improvement program remains to be determined. It would appear that viability of seed as manifested by germination capacity might be considered in the selection of parental material. The effect of seed size upon vigour of 150-day seedlings is in agreement with results for similar studies on other plants (15, 18, 23). It remains to be determined, if the additional vigour of caragana seedlings attributable to large seed size would gradually disappear with approaching maturity as reported for some plants (18, 23), or if the effect is permanent.

First inbred generations [S_1] of alfalfa have been reported (13, 16, 30) to average 68 to 84 per cent of the open-pollination progenies by weight, with a range of 26 to 105 per cent. S_1 progenies of caragana trees in this

study averaged 70 per cent of the open-pollination progenies by weight for 150-day seedlings, with a range of 37 to 100 per cent. The ratio for height of self- and open-pollination seedlings of corn has been reported (7) as 87 : 100; while height ratio for 150-day seedlings of caragana in this study was found to be 80 : 100. Data presented in this study suggest that inbreeding of *C. arborescens* may reduce plant vigour similar to that resulting from the inbreeding of such cross-pollinated plants as alfalfa and corn.

Comparison of S_1 seedlings of 14 caragana trees [Table 10] revealed progenies of three parental trees (21–27, 16–4, V–12] to be superior in mean height to those of all except four trees. The presence of favorable growth factors for two of the same three superior trees [21–27, V–12] was also suggested by the superior vigour demonstrated by their open-pollination progenies. However, there are three possible objections to evaluating the combining ability of parental trees in this study by the vigour of their open-pollination seedlings. These are: first, the degree to which natural cross-pollination as opposed to natural self-pollination has contributed in the production of such progenies is unknown; second, random cross-pollination by the same pollen mixture is not obtained due to the different locations of parental trees; finally, there exists the possibility that the vigour of one-year-old seedlings may not be a valid estimate of the vigour of mature plants.

CONCLUSIONS

It seems that certain conclusions may be drawn from this study of seedling progenies of *Caragana arborescens*. As a result of the large differences in height obtained between seedling progenies, it would appear that the parental trees in this study were genetically different for growth factors determining vigour. Thus it seems evident that selection and hybridization within the species should lead to the production of more valuable commercial types. In the production of vigorous open-pollination progenies, it may not be essential to consider the fertility classification of trees producing the seed. Recent investigations have indicated that vegetative propagation of the species is a relatively simple procedure with softwood cuttings. These characteristics suggest the possibility of adopting the methods of breeding followed for the improvement of alfalfa (30, 31, 32).

SUGGESTIONS FOR FUTURE WORK

From the present study, it is suggested that the alfalfa method of breeding may be applied to *Caragana arborescens* in the following manner. First, selections whose progenies have demonstrated superior vigour in this study should be evaluated as to their general combining ability. It is suggested that such selections be propagated vegetatively and established both in a clonal observation nursery and a polycross nursery. While waiting for the above plantings to attain sexual maturity, further selections should be made from all possible sources. This would ensure a large reservoir of potential material with genetic diversity for future evaluation. Plants in the clonal observation nurseries may be studied in detail, and undesirable clones eliminated with a minimum of labour. Clones exhibiting desirable characteristics [i.e. vigour, fertility, etc.] and having a similar period of bloom would be selected for polycross tests. Second, seed of each of the selected clones as produced in polycross nurseries would be used

for progeny testing of the clone. In this way the clones may be classified as to their general combining ability for vigour. Earlier evaluation of the combining ability of selections might be possible, if selections were crossed with two or more tester selections. Use of self-sterile or low self-fertile selections as the female parents [i.e. testers] would reduce the time required for such crossing. Finally, high combining clones would be propagated and established in natural isolated crossing blocks. Such crossing blocks would be expected to produce progeny of superior vigour, after the plants attained sexual maturity. Until that time the original polycross nurseries could serve as sources for improved commercial seed.

SUMMARY

1. The characteristics of 14 open-pollination selections of *Caragana arborescens*, Lam., (Siberian pea-tree) were investigated, and their relationship to vigour of seedling progenies resulting from self- and open-pollination has been determined.

2. Number of seeds harvested per flower tripped provided a more reliable and consistent measure of self-fertility for parental trees than did the number of pods set per flower tripped. Self-fertility of the 14 trees ranged from 0.37 to 2.51 seeds/flower.

3. Parental source of seed had a significant effect upon both germination capacity and speed.

4. Size of seed showed no relationship to the degree of "set" resulting from either self- or open-pollination. Size of seed failed to exhibit differences in either germination speed or capacity; although for the large size, self-pollination seed exhibited superior germination to that of open pollination seed. Seedlings from the large seed were more vigorous than those from smaller seed. The relationship between weight of seed and seedling height was significant for some trees.

5. Two measures of vigour, height and weight, gave comparable evaluations for 150-day seedlings of caragana, as indicated by a correlation value of .960.

6. First inbred generations of 14 trees ranged from 37 to 100 per cent of the respective open-pollination progenies by weight, for an average vigour ratio of 70 : 100. Height of inbred progenies ranged from 48 to 97 per cent of that for open progenies, for a vigour ratio of 80 : 100. Significant differences for seedling vigour were found between the self-pollination progenies of some parental trees as well as between open-pollination progenies of some trees. Thus, it was evident that parental trees in this study were genetically different for factors affecting vigour of their 150-day seedling progenies.

7. Self-fertility of parental trees was not associated with germination speed or germination capacity for either self- or open-pollination seed. Height of 150-day open-pollination progenies demonstrated no relationship to the self-fertility of their parental trees. However, there were indications that vigorous open-pollination progenies are produced by some low and some medium self-fertile trees.

8. It was apparent that a breeding program for vigour should be effective in the species, and tentative procedures for the improvement of vigour in *Caragana arborescens* are suggested for further study.

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THE BIOLOGICAL ASSESSMENT OF THE VALUE OF RAPESEED OIL MEAL AS A DIETARY COMPONENT[1]

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INTRODUCTION

The acreage sown to rapeseed in Canada has declined markedly from the maximum reached during and immediately following World War II and consequently the oil meal by-product has not been available in quantity for livestock feeding. Should there be a renewed demand for rapeseed oil the utilization of the oil meal would then become a factor in agricultural economy. With this in view a preliminary investigation has been made of methods for the removal or neutralization of the known anti-thyroid activity of the meal. In addition a limited study has been made of feed economy employing laboratory animals. The data obtained may be useful in the consideration of rapeseed oil meal as a component of livestock rations.

Recorded information on the use of rapeseed oil meal[4] in livestock rations is not extensive. The evidence suggests that this material has a fairly high order of digestibility but is not particularly palatable. In Germany it was found that extracted rapeseed meal and rapeseed cake were consumed fairly readily by cows when fed dry up to 2 to 3 kg./day but were refused when moist (1). Local results show that lambs may be conditioned to consume one-quarter pound rapeseed meal daily. Steers will tolerate it in amounts as high as 25 per cent of the grain component of the ration while pigs will consume a ration containing 15 per cent. Metabolism studies with lambs receiving rape showed a dry matter digestibility of the order of 86 per cent (2). Local trials with steers showed a mean digestibility coefficient of 78 per cent. Some information is available on the influence of rapeseed meal on growth of poultry. Inconsistent effects have been reported when rapeseed meal was fed to chicks in the amount of 10 per cent. Normal growth was found when this component replaced sunflower seed oil meal in chick starter ration (3) but retarded growth and lowered feed efficiency have also been reported (4). No significant difference in average gain was obtained when turkeys were fed 25 per cent rapeseed meal or commercial meat meal. Fifteen per cent rapeseed oil cake meal gave significantly better gains than 25 per cent when tested in a 21-day finishing period with cockerels although both gave lower gains than low protein meat meal.

The goitrogenic effect of the members of the genus *Brassica* appears to have been well established. Kennedy and Purves (6) showed that *Brassica* seeds produce thyroid hyperplasia when fed to rats. Blakeley and Anderson (7) demonstrated that 4, 10, and 20 per cent rapeseed oilcake meal when fed in place of meat meal to day-old poults for 42 days produced

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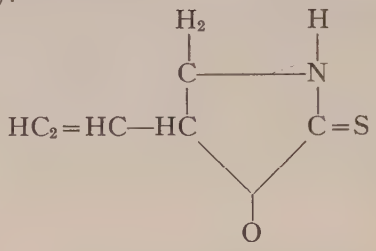
[4] In the interests of brevity, the expression "rapeseed meal" is used subsequently to designate the meal after oil has been extracted.

thyroids 2, 3.2 and 4.8 times normal. It was found (8) that the activity of the crystalline principle of rape was slightly less than that of thiourea when both were fed to rats at 0.5 per cent of the ration.

The goitrogenic principle of rape was shown (9) to be present in the amount of 2 gm./kg. in the seed and was identified as L-5-vinyl-2-thiooxazolidone. The compound appears to be present in the plant as a water-soluble, ether-insoluble precursor from which the thiooxazolidone is formed apparently by enzymatic action. The thiooxazolidone has been obtained in crystalline form and the following structure has been confirmed by Astwood *et al.* (9):



L—5—VINYL—2—THIOOXAZOLIDONE

Carroll (8) also isolated the anti-thyroid compound and established its empirical formula. The compound has recently been synthesized by Ettlinger (10).

Several attempts at removal or neutralization of the anti-thyroid compound have been recorded. Kennedy and Purves (6) reported that the administration of 1000 μg. iodide daily did not completely prevent the development of thyroid hyperplasia in rats fed on *Brassica* seeds. Purves (11) found that iodide and diiodotyrosine were equally effective on the basis of iodine content in moderating the goitrogenic effect but they did not abolish the action even in large doses; dl-thyroxine in daily doses of 3 μg./100 gm. body weight abolished thyroid hyperplasia and produced a hypoactive gland. Wagner-Jauregg and Koch (12) reported that the goitrogenic substance of another member of the *Brassica* genus was destroyed by ordinary cooking. Steamed rapeseed showed no activity when fed to rats for thirty days at the 50 per cent level (Hercus and Purves (13)).

In the feeding trials to be reported measurements were made of the goitrogenic effect of different levels of rapeseed meal. Methods for the removal of the goitrogen or for neutralizing the active principle are described. Comparisons with respect to feed efficiency were made between basal diets and those containing a relatively high proportion of the meal.

EXPERIMENTAL

Diets

The basal diet shown in the first column of Table 1 was used in most of the experiments to be reported. It is a modification of the A.O.A.C. chick diet for vitamin D assay (Methods of Analysis, 5th Ed., 371-372. 1940) with an increase in the amount of calcium and the use of iodine-free salt. One per cent of a mixture of fish and corn oils was incorporated to ensure the presence of sufficient vitamins A and D.

TABLE 1.—COMPOSITION AND ENERGY VALUES OF CHICK DIETS

Ingredient	Basal diet	Modified[1] basal diet	High level rapeseed meal diet
	%	%	%
Yellow corn	57.3	46.0	42.6
Wheat middlings	24.7	28.2	18.4
Casein	11.8	19.3	8.8
Brewer's yeast	2.0	2.2	1.5
$Ca_3(PO_4)_2$	2.0	2.0	2.0
NaCl	1.0	1.0	1.0
$CaCO_3$	0.25	0.25	0.25
Corn oil	0.75	0.75	0.75
Fish oil[2]	0.25	0.25	0.25
$MnSO_4$	9 gm./cwt.	9 gm./cwt.	9 gm./cwt.
Rapeseed meal	—	—	24.6
Digestible protein[3]	16.5	21.0	19.0
Productive energy[3]	949 Cal./lb.	918 Cal./lb.	923 Cal./lb.

[1] Modified to equalize crude protein content of rapeseed meal diet.
[2] The fish oil had a potency of 2400 units A and 400 units D per gram.
[3] Calculated from data of Fraps (14).

Experimental diets consisted of basal ration and varying levels of rapeseed meal. Even low level diets proved to be unpalatable for the rat but the chick was found to be a satisfactory test animal.

For purposes of comparison the percentage composition and energy values of the basal ration and a modified basal ration used in one trial are shown with the high level rapeseed meal ration in Table 1.

Demonstration of Goitrogenic Effect

One-day-old chicks were placed on diets consisting of basal ration admixed with 25, 15 and 10 per cent rapeseed meal. At the end of a 4-week treatment period the thyroid glands were exercised and their weights recorded. The goitrogenic activity of the rapeseed meal was expressed in terms of thyroid gland hyperplasia (gland weight in mg.) calculated on the basis of 100 gm. body weight (or T/W ratio). These data (Experiment 2) are combined with similar data from later trials with different breeds and are presented in Table 2.

It is evident that rapeseed meal had a goitrogenic effect on the chick and that an upward gradation of thyroid response occurred with increasing dietary concentration of the active compound. In the majority of cases the rapeseed meal diets produced heavier birds than did the control diets and, in general, the increased weights were correlated with feed efficiency. The feeding of lower levels of the meal appeared to be advantageous. The results may have been influenced by the fact that the protein levels of the various diets were not identical. This point will be discussed later.

Removal of the Goitrogenic Principle

The goitrogenic activity of the meal having been demonstrated, the removal of the active principle was attempted. Following the report of Astwood *et al.* (9) that the active principle in rapeseed is present as a water-soluble precursor, cold water extracted rapeseed meal was prepared and

TABLE 2.—RESPONSE OF CHICKS TO RAPESEED OIL MEAL

Expt. No.	Breed and sex	Treatment[1]	No. of birds	Av. body wt. (gm.)	Av. T/W (mg./100 gm.)	Av. feed/gain (gm./gm.)
2	NH × BR	Basal	14	170	18.2	3.16
	Female	25% rape	12	181	159.7	3.56
		15% rape	13	233	111.3	2.95
		10% rape	13	216	101.7	2.82
4	NH × BR	Basal	12	218	10.0	2.01
	Female	25% rape	10	187	133.2	2.86
7	WL × NH	Basal	31	193	11.7	2.47
	Male and female	25% rape	31	253	51.9	2.04
8	WL	Basal	30	178	20.2	2.58
	Male and female	25% rape	20	224	24.3	2.37
11	NH × BBW	Basal[2]	30	247	6.6	3.11
	Male	25% rape	30	301	39.5	2.50

[1] Rapeseed oil meal added to basal diet in amounts indicated; crude protein content of diets ranged from 20-25 per cent. Feeding period 4 weeks.

[2] Crude protein was equalized with rapeseed meal ration for this experiment.

TABLE 3.—EFFECT OF WATER AND HEAT TREATMENT OF RAPESEED OIL MEAL

Expt.[1] No.	Breed and sex	Treatment[2]	No. of birds	Av. body wt. (gm.)	Av. T/W (mg./100 gm.)	Av. feed/gain (gm./gm.)
3	NH × BR	Basal	14	178	15.3	2.02
	Female	25% c.w. extracted	14	167	103.1	2.12
		15% c.w. extracted	14	192	80.5	2.01
		10% c.w. extracted	14	184	69.9	1.83
4	NH × BR	Basal	12	218	10.0	2.01
	Female	25% unheated	10	187	133.3	2.86
		25% heated	12	154	112.2	3.13
5	NH × BR	Basal	11	299	11.0	2.84
	Female	25% h.w. extracted	11	277	18.1	3.09
		15% h.w. extracted	11	352	16.9	2.56
		10% h.w. extracted	9	356	14.8	2.36
8	WL	Basal	30	178	20.2	2.58
	Male and female	25% h.w. extracted	29	216	22.3	2.51
9	WL	Basal	30	216	8.4	2.41
	Male	25% h.w. extracted	27	212	13.4	2.49
10	NH × BBW	Basal	16	255	9.0	2.56
	Male	25% h.w. extracted	29	280	15.0	2.26

[1] Three-week treatment in Experiment 3; 4-week treatment in other experiments.

[2] Rapeseed oil meal unheated, heated, extracted with cold water (c.w.) or with hot water (h.w.) added to basal diet in amounts indicated; protein content of diets ranged from 20-25 per cent.

made up at the same dietary levels as those previously tested. The results (Experiment 3 in Table 3) demonstrate that cold water extraction, under the conditions of this experiment, was ineffective in removing the goitrogen.

The effectiveness of dry heat in moderating the goitrogenic effect was tested. The meal was heated in an air oven at 115° C. for 24 hours, then

admixed with basal ration to form the experimental diet. The results (Experiment 4 in Table 3) show that dry heat caused only a slight reduction in the goitrogenic effect of the meal.

Following reports that boiling or steaming of rapeseed meal destroyed the goitrogenic activity, hot water extraction was investigated. The meal was placed in a large container with water and steam was introduced until the temperature reached 98°-99° C. The hot extract was decanted and the process was repeated several times. Five-day-old chicks were placed on diets containing 25, 15 and 10 per cent of this dried meal and the thyroid response was measured after four weeks of treatment. In comparing these results with those from Experiment 4, Table 3, employing the same breed and sex of chick, it is evident that the hot water treatment has been very effective in removing the goitrogen although a statistically significant treatment difference ($P < 0.01$) is still apparent. The lower levels of rapeseed meal improved feed efficiency and produced heavier birds than the 25 per cent level.

Neutralization of the Goitrogenic Principle

Reports by Kennedy and Purves (6) and Purves (11) concerning the effect of iodide on thyroid hyperplasia are conflicting. To test the effectiveness of iodide as a neutralizing factor, diets were prepared which contained 25 per cent rapeseed meal and 0.003, 0.006 or 0.012 per cent iodine added as potassium iodide. Birds were sexed at the time of thyroid removal for determination of a possible sex difference in reaction. The data for this two-week trial (Experiment 6) are presented in Table 4.

TABLE 4.—EFFECT OF DIETARY IODINE ON GOITROGENIC ACTION OF RAPESEED OIL MEAL

Treatment	No. of birds	Av. body wt. (gm.)	Av. T/W (mg./100 gm.)	Av. feed/gain (gm./gm.)
Expt. 6 2-week treatment. WL mixed sexes—				
Basal	30	103	12.1	2.95
25% rape	30	97	53.9	2.99
25% rape + 0.003% I_2	25	105	17.5	2.88
25% rape + 0.006% I_2	24	115	20.5	2.40
25% rape + 0.012% I_2	29	119	19.6	2.26
Expt. 7 4-week treatment. WL × NH mixed sexes—				
Basal	31	193	11.7	2.47
25% rape	31	253	51.9	2.04
25% rape + 0.0015% I_2	31	247	21.7	2.53
25% rape + 0.0030% I_2	32	262	20.8	2.48
Expt. 11 4-week treatment NH × BBW males—				
Basal[1]	30	247	6.6	3.11
25% rape	30	301	39.5	2.50
25% rape + 0.0015% I_2	30	270	13.3	2.54
25% rape + 0.00075% I_2	30	263	11.5	2.86

[1] The basal ration for this experiment has been adjusted to a higher protein level than the previous control rations.

Iodide at the levels employed was completely effective ($P < 0.01$), in counteracting the goitrogenic effect of 25 per cent rapeseed meal in the diet. Differences in response between the iodide levels used in this experiment were not significant. A significant sex difference in response to rapeseed meal was evident ($P < 0.01$), females showing greater sensitivity to the goitrogen.

The effectiveness of iodide against the cumulative effect of the goitrogen was tested in a four-week trial (Experiment 7). One-day-old chicks of mixed sexes were placed on diets containing 0.003 and 0.0015 per cent iodine as potassium iodide and the results obtained are presented in Table 4. Iodide in the amounts employed did not completely prevent thyroid hyperplasia ($P < 0.05$) but obviously had an inhibitory action.

Severe and unexpected losses occurred in two further trials. In these cases the rapeseed meal was drawn from an isolated lot and evidently contained a "toxic" substance since mortality was as high as 50 per cent in some groups. Birds surviving after four weeks of treatment had failed to gain normal weight, showed marked leg weakness and had nodular thyroids. It was interesting to note that the hot water treatment of the meal prevented the appearance of these symptoms and that the inclusion of iodide appeared to alleviate them and to reduce the death rate. Microscopical examination of the meal failed to reveal the source of "toxicity". No similar difficulty was encountered with a fresh supply of meal.

Throughout these trials birds fed rapeseed meal showed greater weight gains and feed efficiency than control birds and it was thought that the higher protein level of the former ration might have been responsible for these effects. In order to test this possibility the crude protein levels of the diets were equalized at approximately 25 per cent by adjusting some of the components of the basal ration. The mineral constituents, with the exception of iodide, were also equalized. The effects of these rations, with and without added iodide, are shown in Table 4 under Experiment 11.

The data show that the previously observed feed efficiency was not due to differences in protein concentration since lower feed : gain ratios for rape-fed birds were still obtained when the crude protein levels were identical. Potassium iodide at both of the levels employed was very effective ($P < 0.05$), in counteracting the goitrogenic effect of the rapeseed meal. However, since the goitrogenic effect of a 25 per cent rapeseed meal diet in this experiment was not as great as in earlier experiments employing other breeds of chicks, no prediction can be made of the effectiveness of these levels under different experimental conditions. It can be concluded, however, from this and the above experiments, that dietary iodide in low, non-toxic amounts will largely counteract the goitrogenic effect of rapeseed meal.

DISCUSSION

The data show wide breed differences in thyroid response to rapeseed meal diets but these differences were no greater than when thiouracil was fed in parallel tests. A continuing supply of chicks of the same breed and preferably of the same sex would have been desirable but these conditions could not be met during this investigation.

It may be observed that, in general, the rapeseed meal diets produced heavier birds than the corresponding controls. This was true whether the meal was admixed in the ration in its original form or after removal or neutralization of most of the goitrogen.

Some consideration was given to the possibility that the higher level of protein in the rape diet might have had an influence on the observed feed efficiency but when the control and experimental diets were at the same crude protein level the superior effect of the rapeseed meal was still apparent. Actually the modified basal ration contained a higher proportion of digestible protein than the rape ration (Table 1). Thus it would appear that the amount of protein present does not explain the feed efficiency obtained with the rapeseed meal. Consideration was also given to the possibility that a difference in energy values might be the factor responsible for the differences in feed efficiency. However, calculation of the productive energy values of the diets according to the data of Fraps (14) has shown that the diets are practically equal in caloric value and well above the accepted requirement for growth. Since neither hot water extraction nor the addition of iodide was completely effective in counteracting the goitrogenic effect of the meal it is possible that a lowered basal metabolic rate is the factor responsible for the observed feed efficiency. A consideration of the quality of rape protein cannot be dismissed as a possible factor.

Although the feed efficiency obtained in these four-week trials with rapeseed meal is obviously advantageous the continued use of such a potent goitrogenic material might be harmful. However, since feed efficiency was still obtained with rapeseed meal which had been extracted or which contained added iodide, the adoption of either of these measures should provide a protein supplement which could be utilized in a long-term feeding program. The investigation is being continued to determine if relatively high levels of rapeseed meal can be efficiently utilized in broiler rations.

SUMMARY

1. The young chick is highly responsive when fed for four weeks on rations containing rapeseed oil meal. This response is characterized by marked thyroid hyperplasia and accelerated body weight gain accompanied by feed efficiency.

2. The degree of response differs with the breed of chick employed.

3. A marked inhibition of goitrogenic activity may be obtained either by hot water extraction of the meal or by the addition of iodine in the form of inorganic iodide.

4. The findings suggest that rapeseed oil meal could be utilized to advantage in two ways:

(*a*) As a protein supplement in the ration of growing chicks after either hot water extraction or the addition of iodide.

(*b*) In its original form, as a protein supplement and fattening agent for chicks approaching market weight.

Livestock might also be expected to respond favourably to rations containing rapeseed oil meal but optimum feeding conditions would have to be established for each species.

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